



Original Research Article

Solvent-free mechanochemical green synthesis of Schiff bases of tranexamic acid and study of their urease inhibitory and antioxidant activities

Abid Zulfiqar^{a,b} , Dildar Ahmed^{a*} ^a Department of Chemistry, Forman Christian College Lahore, Pakistan^b Present affiliation: INM-Leibniz Institute for New Materials, Campus D2-2, 66123 Saarbruecken, Germany

ARTICLE INFORMATION

Received: 23 January 2022

Received in revised: 12 February 2022

Accepted: 16 February 2022

Available online: 12 March 2022

DOI: 10.22034/ajgc.2022.1.4

KEYWORDS

Tranexamic acid derivatives

Bioactivities

Green chemistry

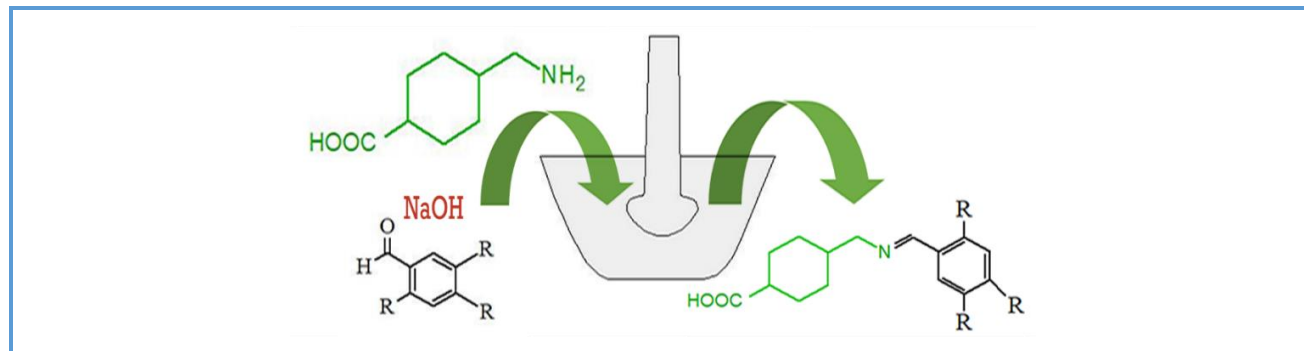
Grinding method

ABSTRACT

Three Schiff bases of tranexamic acid were successfully synthesized by a mechanochemical green method, which is 4-((5-bromo-2-hydroxybenzylidene)amino)methyl)cyclohexanecarboxylic acid (SB1), 4-((2-hydroxybenzylidene)amino)methyl)cyclohexanecarboxylic acid (SB2), and 4-((4-nitrobenzylidene)amino)methyl)cyclohexanecarboxylic acid (SB3) in good yield. Their structures were confirmed based on spectroscopic data. The bases displayed considerable urease inhibitory activity with IC_{50} ($\mu\text{g/mL}$) 33.41, 40.64, 26.18, and 11.14 for SB1, SB2, SB3, and standard thiourea, respectively. They revealed weak antiradical activity in DPPH assay with EC_{50} ($\mu\text{g/mL}$) 1138, 1239, and 24248 for SB1, SB2, and SB3, respectively. In conclusion, grinding is an efficient and environmentally friendly method for synthesizing these bases, which may provide potential candidates for new medicines.

© 2022 by SPC (Sami Publishing Company), Asian Journal of Green Chemistry, Reproduction is permitted for noncommercial purposes.

Graphical Abstract



Introduction

Tranexamic acid (4-(aminomethyl)cyclohexanecarboxylic acid) is a chemical compound having an amino as well as a carboxylic group like an amino acid. It is a synthetic derivative of the amino acid lysine. It has anti-fibrinolytic activity and is used as a medicine to reduce excessive bleeding [1]. Tranexamic acid is a colorless solid soluble in water but insoluble in ethanol and methanol at neutral pH.

The aldehydes used as reactants here included 5-bromo-2-hydroxybenzaldehyde, 2-hydroxybenzaldehyde, and 4-nitrobenzaldehyde. As the literature reveals, derivatives of tranexamic acid have been synthesized by some research groups. These include *N*-acetyltranexamic acid, *N*-phthaloyltranexamic acid, di-*N*-phthaloyltranexamate diaquocopper (II), di-*N*-acetyltranexamate diaquocopper (II) and di-tranexamate diaquo copper (II) [2], carbamic acid salts of tranexamic acid esters [3], and vanadium complexes of Schiff bases derived from tranexamic acid with 2-fluorobenzaldehyde and furfuraldehyde [4]. A Schiff base of tranexamic acid with 5-bromo-2-hydroxybenzaldehyde was synthesized using the conventional reflux method [5], and another Schiff base of tranexamic acid was synthesized with 2-hydroxybenzaldehyde [6]. Organotin(IV) complexes of 4-(((E)-(2-hydroxyphenyl)methylidene)amino)methyl)cyclohexane carboxylic acid was prepared by the reaction of di and triorganotin salts using water and ethanol as solvents [6].

In contrast to the conventional solution-based reactions, mechanochemical green synthesis is an environmentally benign method that, in general, gives a higher yield of the synthesized products and uses no toxic organic solvent. Because of these advantages, this new

strategy should be given more attention for organic synthesis.

Some biological activities of tranexamic acid derivatives have also been studied, including transdermal permeation enhancement, antibacterial, and antifungal [3, 4, 6]. During the last several years, much work has been done on the formation of Schiff bases and their bioactivities. Most of the Schiff bases are active against fungal and bacterial strains and show antioxidant and enzyme inhibition activities.

Many Schiff bases have also been shown to have considerable urease inhibitory activity. Urease is a metalloenzyme with nickel-metal ion as a cofactor [7]. It catalyzes the hydrolysis of urea into ammonia and carbamate, which spontaneously produces carbonic acid and another molecule of ammonia. The production of ammonia results in an increase in pH. A bacterium *Helicobacter pylori* use this reaction to survive in the acidic environment of the human stomach. The ammonia produced by the reaction neutralizes the acidic medium around the bacterium and, thereby, makes the microorganism's survival possible, which grows and damages the stomach wall, causing peptic ulcers. The ulcer may sometimes grow into cancer. When urea is used as a fertilizer in agriculture, it produces ammonia upon urease catalysed catalyzed hydrolysis. This has environmental as well as economic consequences [8, 9].

Antioxidants have several applications. They are used as food preservatives to inhibit rancidity. In medicine, they are used to control or treat disorders caused by oxidative stress or excessive production of reactive oxygen species (ROS) in our body [10]. It has been proposed that antioxidants work through the HAT (hydrogen atom transfer) or SET (single electron transfer) mechanism [11]. Several analytical methods are used to analyze the antioxidant potential of a substance [12]. One

such method is the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. It gives violet-blue color in solution which changes into pale yellow color upon acceptance of an electron or hydrogen atom. The change can be quantitatively monitored by measuring the absorbance at 517 nm [8].

The objective of the present study was to explore the viability of synthesizing Schiff bases of tranexamic acid through the solvent-free mechanochemical green method and to determine urease inhibitory activity and antioxidant potential of the synthesized compounds. The increasing awareness of the environmental impact of chemical activities demands exploring new methods for the synthesis of chemical compounds, the methods that are environmentally friendly and economically more viable. The solvent-free grinding method has many advantages over the conventional reflux method, as it uses no organic solvent and external heating. It has higher yields. For example, the in-solution synthesis of Schiff bases is a reversible reaction. In the grinding method, the side product water evaporates due to the heat produced in the grinding process, so the backward reaction is hindered, resulting in a higher yield.

Experimental

Materials & methods

Tranexamic acid was purchased from Munawar Pharma (Lahore, Pakistan). 5-Bromo-2-hydroxybenzaldehyde, 2-hydroxybenzaldehyde, 4-nitrobenzaldehyde, urease, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Steinheim, Germany). Glacial acetic acid, potassium monophosphate, dipotassium phosphate, sodium hypochlorite, sodium salicylate, and sodium phosphate were purchased from Daejung (Siheung City, Korea),

and urea, ethylenediaminetetraacetic acid (EDTA), and thiourea were purchased from BDH Labs (Cambridge, England). The progress of the reaction was monitored by thin-layer chromatography (TLC). All synthesized compounds (SB1-SB3) were characterized by IR, ^1H NMR, ^{13}C NMR spectroscopy. IR spectra were recorded on a FT-IR, Agilent Technologies Cary 630. NMR (600 MHz) spectra were run on a Bruker-Avance III HD instrument.

General procedure for the synthesis of compounds SB1-SB3

2 mmol tranexamic acid and 2 mmol aldehyde were mixed in a mortar. To this mixture, 0.14 g NaOH compound was added. The mixture was ground with the help of a pestle for 2/3 hours without using any solvent. The progress of the reaction was monitored with TLC (solvent system, hexane: ethyl acetate, 4:1). After completion of the reaction, the product was dissolved in methanol and neutralized by adding a few drops of hydrochloric acid (12 M). The solution was filtered using Whatman filter paper No. 1 under gravity. The filtrate was collected in a china dish. The solvent was allowed to evaporate in a fume hood at room temperature. As a result, the product was obtained as an amorphous powder. Synthesized Schiff bases are shown in Table 1.

Urease inhibitory assay

Urease inhibitory activities of the synthesized Schiff bases were determined using the method reported by Weatherburn (1967) [13]. A buffer solution was prepared by mixing equal volumes of 100 mM urea, 0.01 M K_2HPO_4 , 1 mM EDTA, 0.01 M LiCl_2 , and pH was adjusted to 8.2 with 25% aqueous KOH. An alkali reagent was prepared by adding equal volumes of 0.5% w/v NaOH and 0.1% active chloride NaOCl. The phenol reagent was prepared by adding equal

volumes of 1% w/v phenol and 0.005% sodium nitroprusside. The enzyme solution was prepared by dissolving its 5 units (12.4 mg) in 100 mL distilled water. Based on trial tests, different dilutions of the synthesized Schiff base were prepared in DMSO, which were 5, 10, 15, 20, 25, 30, 35, 40, 50 µg/mL. Thiourea was used as a positive control. Its dilutions consisted of 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 µg/mL.

In 96-well plates, 10 µL enzyme solution and 10 µL sample solution in 40 µL buffer solution were added. After the 30 min incubation period at 37 °C, 40 µL phenol and 40 µL alkali reagents were added to each well and again incubated at 37 °C. After incubation of 30 min, absorbance was noted at 625 nm by a microplate reader. All sample tests were performed in three replicates to obtain mean values. The urease inhibitory activity was determined by using the following formula:

$$\text{Inhibitory Activity (\%)} = [(1 - (A_s / A_c))] \times 100$$

Where, A_s is the absorbance of the sample (10 µL enzyme solution + 10 µL sample solution in 40 µL buffer solution, 40 µL phenol reagent, and 40 µL alkali reagent); A_c is the absorbance of the control (10 µL enzyme solution + 10 µL DMSO in 40 µL buffer solution, 40 µL phenol reagent and 40 µL alkali reagent).

Antioxidant assay

To assess the antioxidant potential of the synthesized compound, DPPH radical scavenging assay was used according to the protocol of Brand-Williams *et al.* (1994) using a reported procedure [14]. A stock solution of DPPH radical was prepared with a concentration of 0.4 mM (100 mL) by dissolving 0.0158 g DPPH in methanol. The working solution of DPPH was prepared by diluting 5 mL DPPH stock solution with methanol to achieve

the absorbance of about 0.97 (\pm 0.03) at 517 nm (around 30 mL solvent was required). Based on trial tests, different dilutions of the synthesized Schiff bases were prepared in DMF, which were 500, 600, 700, 800, 900, 1000, 1500, 2000 µg/mL. Ascorbic acid was used as a positive control. A measured volume (0.4 mL) of a sample dilution was taken in a test tube followed by the addition of a 3 mL DPPH working solution. The mixture was incubated for 30 min at 37 °C. The absorbance of the solution was noted at 517 nm. The antioxidant activity was calculated as per the following equation.

$$\text{Antioxidant Activity (\%)} = [(1 - (A_s / A_c))] \times 100$$

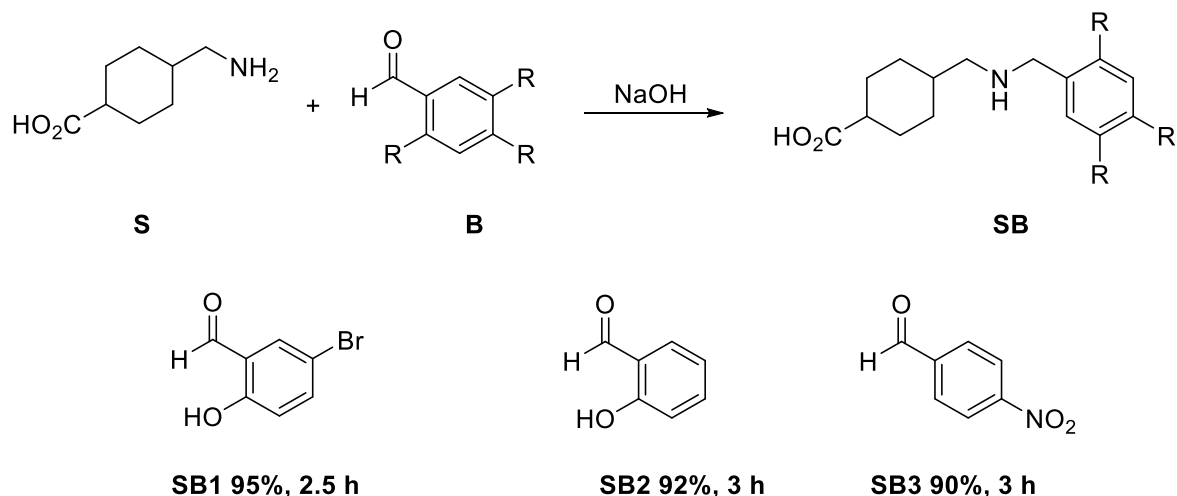
Where, A_s and A_c are the absorbance of the sample (3 mL DPPH working solution and 0.4 mL sample dilution), and control (3 mL DPPH working solution and 0.4 mL DMF), respectively. A mixture of 3 mL methanol and 0.4 mL DMF was used as a blank.

Results and Discussion

Elucidation of structures

Three Schiff bases of tranexamic acid, SB1, SB2, and SB3, were successfully synthesized by the grinding method (Scheme 1). The color was SB1 yellow, while SB2 and SB3 were brown. The synthesized compounds were characterized by FT-IR, ^1H NMR and ^{13}C NMR analyses.

Their FT-IR spectral data are displayed in Table 1, along with the interpretation of the peaks. The successful synthesis of all compounds was observed by forming an imine group (=CH) that gives an absorption peak around 1600-1620 cm^{-1} . Other absorption peaks in Table 1 are referred to as reactants because aldehyde and amine groups from both reactants were used to participate in the reaction to form the imine bond.

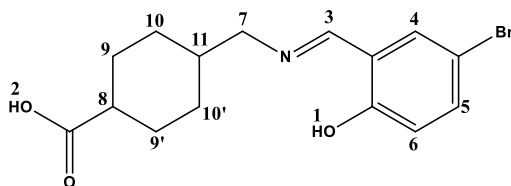


Scheme 1. Solvent-free synthesis of Schiff bases of tranexamic acid by grinding method

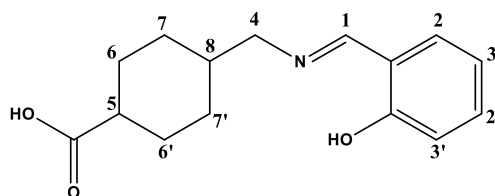
The ^1H NMR and ^{13}C NMR spectral data are shown in Tables 1. The ^1H NMR spectrum of SB1 was obtained in deuterated dimethyl sulfoxide (DMSO). The peaks for protons 1 and 2 were observed at 12.15 and 11.40 ppm, which represents carboxylic and phenolic groups. The ^1H NMR spectra of SB2 and SB3 were recorded in deuterated water (D_2O). The peaks of the protons from carboxylic and phenolic

groups are missing in the ^1H NMR spectra of SB2 and SB3. These groups can be deprotonated in the presence of a polar solvent during the ^1H NMR analysis [15]. The peak around 8.30-8.50 in all compounds in Table 1 indicated the presence of the imine group. The coupling of different protons can be observed from the coupling constants.

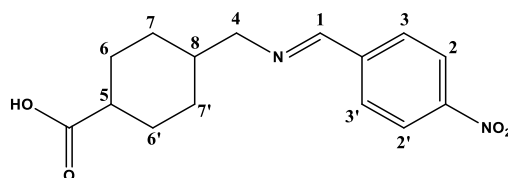
Table 1. FT-IR, ^1H NMR and ^{13}C NMR analyses for synthesized compounds SB1, SB2 and SB3



Compound SB1: IR (KBr) (ν_{max} / cm^{-1}): 3367 (-OH), 2926 (=CH-Ar), 2857 (- CH_2 -), 1634 (-C=O), 1608 and (-N=C-). ^1H NMR (600 MHz, DMSO): δ 12.15 (s, 1H, H₁), 11.40 (s, 1H, H₂), 8.50 (s, 1H, H₃), 7.657-7.652 (d, 1H, H₄, $J = 3$ Hz), 7.450-7.431 (dd, 1H, H₅, $J = 9$ and 2.4 Hz), 6.83-6.82 (d, 1H, H₆, $J = 9$ Hz), 3.44-3.43 (d, 2H, H₇, $J = 6.6$ Hz), 2.64-2.60 (m, 1H, H₈), 1.86-1.70 (m, 4H, H_{9,9'}), 1.24-1.21 (m, 4H, H_{10,10'}), 0.96-0.94 (m, 1H, H₁₁). ^{13}C NMR (150 MHz, DMSO): δ 180.2 (1C, C₁), 165.0 (1C, C₂), 160.8 (1C, C₃), 135.2 (1C, C₄), 133.8 (1C, C₅), 124.1 (1C, C₆), 120.3 (1C, C₇), 119.9 (1C, C₈), 65.1 (1C, C₉), 42.2 (1C, C₁₀), 38.9 (1C, C₁₁), 31.0 (2C, C_{12,12'}), 30.2 (2C, C_{13,13'}).



Compound SB2: IR (KBr) (ν_{\max} / cm^{-1}): 3374 (-O-H), 2970 (=C-H), 2868 (-CH₂-), 1638 (-C=O), and 1620 (-N=C-). ¹H NMR (600 MHz, D₂O): δ 8.391 (s, 1H, H₁), 8.21-8.19 (d, 2H, H_{2,2'}, J = 9 Hz), 7.92-7.90 (d, 2H, H_{3,3'}, J = 9 Hz), 2.75-2.74 (d, 2H, H₄, J = 7.2 Hz), 2.03-2.00 (m, 1H, H₅), 1.862-1.739 (m, 4H, H_{6,6'}), 1.315-1.236 (m, 4H, H_{7,7'}), 0.996-0.927 (m, 1H, H₈). ¹³C NMR (150 MHz, DMSO): δ 180.2 (1C, C₁), 165.1 (1C, C₂), 160.8 (1C, C₃), 132.9 (1C, C₄), 131.9 (1C, C₅), 122.5 (1C, C₆), 120.9 (1C, C₇), 118.0 (1C, C₈), 65.1 (1C, C₉), 42.2 (1C, C₁₀), 33.9 (1C, C_{11,11'}), 29.0 (2C, C_{12,12'}), 28.1 (2C, C_{13,13'}).



Compound SB3: IR (KBr) (ν_{\max} / cm^{-1}): 3275 (-O-H), 2908 (=C-H), 2868 (-CH₂-), 1670 (-C=O), 1620 (-N=C-), and 1564 (N-O). ¹H NMR (600 MHz, D₂O): δ 8.30 (s, 1H, H₁), 7.74-7.72 (d, 2H, H_{2,2'}, J = 8.4 Hz), 7.39-7.38 (d, 2H, H_{3,3'}, J = 8.4 Hz), 2.787-2.775 (d, 2H, H₄, J = 7.2 Hz), 2.05-2.01 (m, 1H, H₅), 1.86-1.74 (m, 4H, H_{6,6'}), 1.29-1.24 (m, 4H, H_{7,7'}), 1.0-0.94 (m, 1H, H₈). ¹³C NMR (150 MHz, DMSO): δ 185.7 (1C, C₁), 165.3 (1C, C₂), 149.9 (1C, C₃), 140.3 (1C, C₄), 129.9 (2C, C_{5,5'}), 123.3 (2C, C_{6,6'}), 64.2 (1C, C₇), 43.1 (1C, C₈), 34.1 (1C, C₉), 28.9 (2C, C_{10,10'}), 38.9 (1C, C_{11,11'}).

Urease inhibitory activities

The urease inhibitory activities of the Schiff bases of the tranexamic acid were considerable and dose-dependent. The IC₅₀ values of the activities are shown in Figure 1. As the figure displays, SB3 was most potent, followed by SB1.

Antioxidant activities

All the synthesized Schiff bases showed low antioxidant activities, which were concentration-dependent. The EC₅₀ values of the activity are shown in Figure 2.

The conventional reflux method is generally used for organic synthesis. However, the method suffers from several issues such as poor yield, high cost, and environmental problems.

Like many other reactions, the Schiff bases syntheses are generally shown to have some degree of reversibility when carried out in solution. This affects the yield of the products. Moreover, for in-solution reactions, generally, volatile organic solvents are used, which not only add to the cost of the process are also hazardous for the environment [16]. Keeping these issues in view, the solvent-free mechanochemical method provides a viable alternative [17, 18]. It can be applied by grinding the reactants and is applicable where at least one of the reactants is solid.

In the present work, Schiff bases were synthesized using a mechanochemical green method. The method consisted of grinding with the help of a mortar and pestle. Products were obtained in high yield (90-95%), indicating the

method's efficiency. Since no organic solvent was used in the synthesis, it had other advantages, including environmental sustainability, safety, and cost-effectiveness.

The synthesized compounds showed considerable urease inhibitory activity, SB3 being most potent followed by SB1. Urease inhibitors are required to treat peptic ulcer caused by *H. pylori*. For economic and environmental reasons, they are also needed to slow the urease catalyzed hydrolysis of urea in fertilizer in agricultural forms [8].

As Figure 2 shows, the bases' antioxidant activities were very low compared to the standard ascorbic acid. SB1 was more active than SB2, while SB3 showed very low antioxidant activity and thus was not shown in the Figure 2. A possible reason for the poor antioxidant activities of these compounds might be due to their poor ability to involve themselves in the SET (single electron transfer) or HAT (hydrogen atom transfer) mechanism required for having antioxidant potential [8].

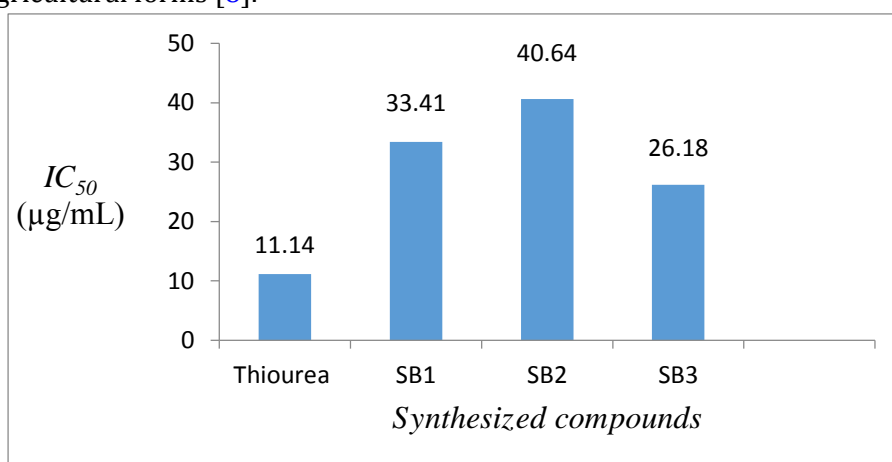


Figure 1. Urease inhibitory activity of the synthesized Schiff bases of tranexamic acid and the standard thiourea ($n = 3$)

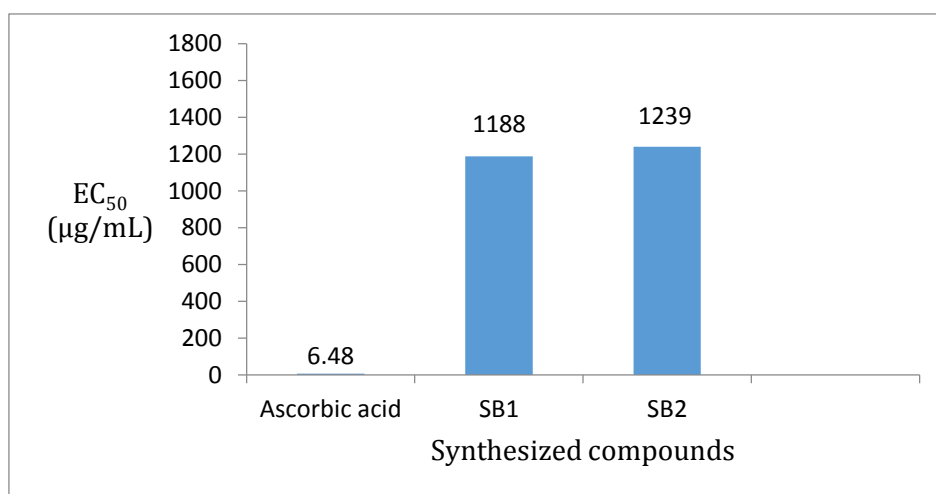


Figure 2. Antioxidant activities of ascorbic acid and synthesized Schiff bases SB1, SB2 according to DPPH assay, SB3 showed almost no activity ($n = 3$)

Conclusions

The solvent-free grinding method was found useful for synthesizing the Schiff bases of tranexamic acid. The yields were in the range of 90-95%, demonstrating the efficiency of the method used. The Schiff bases exhibited considerable urease inhibitory activities, alluding to their possible application to further relevant investigations.

Acknowledgments

The authors thankfully acknowledge the support of the Chemistry Department during this research work.

Disclosure Statement

No potential conflict of interest was declared by the authors.

Orcid

Abid Zulfiqar  0000-0001-9382-5666

Dildar Ahmed  0000-0002-3518-7181

References

- [1]. Shah M.U.A., Asghar M.I., Siddiqi R., Chaudhri M.S., Janjua A.M., Iqbal A. *J. Coll. Physicians Surg. Pak.*, 2015, **25**:161
- [2]. Khan M.F., Khan M.F., Ashfaq M., Khan G.M. *J. Med. Sci.*, 2001, **1**:327
- [3]. Vávrová K., Hrabálek A., Doležal P., Holas T., Klimentová J. *J. Controlled Release*, 2005, **104**:41
- [4]. Shahzadi S., Ali S., Parvez M., Badshah A., Ahmed E., Malik A. *Russ. J. Inorg. Chem.*, 2007, **52**:386
- [5]. Shuja S., Ali S., Khalid N., Parvez M. *Acta Crystallogr. C.*, 2007, **63**:0879
- [6]. Shah F.A., Ali S., Shahzadi S. *J. Iran. Chem. Soc.*, 2010, **7**:59

- [7]. Amtul Z., Rahman A.U., Siddiqui R.A., Chaudhary M.I. *Curr. Med. Chem.*, 2002, **14**:1323
- [8]. Mir H., Ahmed D. *J. Chem. Soc. Pak.*, 2016, **38**:981
- [9]. Aslam M.A., Mahmood S., Shahid M., Saeed A., Iqbal J. *Eur. J. Med. Chem.*, 2011, **46**:5473
- [10]. Kumar S. *Adv. Appl. Sci. Res.*, 2011, **2**:129
- [11]. Muhammad A.J., Ahmed D., Yousuf S., Tabassum N., Qamar M.T. *Heliyon*, 2019, **5**:e01758
- [12]. Iqbal P., Ahmed D., Asghar M.N. *Asian Pac. J. Trop. Med.*, 2014. **7S1**:S473
- [13]. Weatherburn M.W. *Anal. Chem.*, 1967, **39**:971
- [14]. Brand-Williams W., Cuvelier M.E., Berset C. *Lebensm. Wiss. Technol.*, 1995, **28**:25
- [15]. Koeppe B., Guo J., Tolstoy P.M., Denisov G.S., Limbach H.H. *J. Am. Chem. Soc.*, 2013, **135**:7553
- [16]. Tigineh G.T., Wen Y.S., Liu L.K. *J. Tetrahedron*, 2015, **71**:1
- [17]. Sachdeva H., Saroj R., Khaturia S., Dwived D. *Green Process. Synth.*, 2012, **1**:469
- [18]. Junne S.B., Zangade S.B. *E. J. Chem.*, 2012, **4**:23

How to cite this manuscript: Abid Zulfiqar, Dildar Ahmed*. Solvent-free mechanochemical green synthesis of Schiff bases of tranexamic acid and study of their urease inhibitory and antioxidant activities. *Asian Journal of Green Chemistry*, 6(1) 2022, 40-47. DOI: 10.22034/ajgc.2022.1.4