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PII: S1381-1177(15)00065-X
DOI: http://dx.doi.org/doi:10.1016/j.molcatb.2015.03.008
Reference: MOLCAB 3128

To appear in: *Journal of Molecular Catalysis B: Enzymatic*

Received date: 5-12-2014
Revised date: 12-3-2015
Accepted date: 12-3-2015

Please cite this article as: Saima, A.G. Lavekar, R. Kumar, A.K. Sinha, Bovine serum albumin triggered waste-free aerobic oxidative coupling of thiols into disulfides on water: An extended synthesis of bioactive dithiobis(phenylene)bis(benzylideneimine) via sequential oxidative coupling-condensation reactions in one pot from aminothiophenol and benzaldehyde, *Journal of Molecular Catalysis B: Enzymatic* (2015), http://dx.doi.org/10.1016/j.molcatb.2015.03.008

Graphical Abstract:

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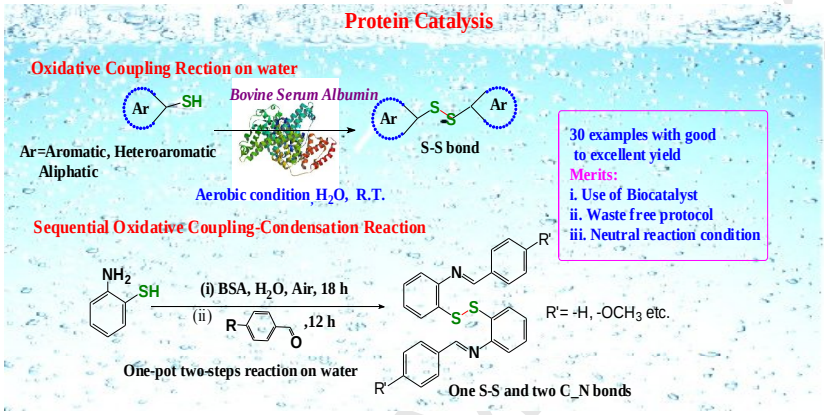

A green and clean route for the aerobic oxidative coupling of thiols including aromatic, heteroaromatic and aliphatic thiols employing BSA as a biocatalyst "on water" has been developed towards formation of (S-S) disulfides under metal/non-metal- or base-free conditions. The methodology also applicable for successful synthesis of diallyldisulfide (DADS), an important precursor of bioactive allicin. Interestingly, bis(aminophenyl)disulfide generated by oxidation of aminothiophenol could directly react with benzaldehyde to form bioactive bis(aminophenyl) disulphidediimines in sequential (oxidation-condensation) manner with one S-S and two C-N bonds in one pot.

Highlights

- A mild and environmentally benign protocol was developed using Bovine Serum Albumin as a catalyst.
- Aerobic oxidation using water as green solvent.
- Broad substrates scope.
- Extended for the synthesis of bioactive bis(aminophenyl)disulphidediimines in one pot from aminothiophenol and benzaldehydes in sequential manner.
- Good recyclability upto four cycles.

Bovine serum albumin triggered waste-free aerobic oxidative coupling of thiols into disulfides on water: An extended synthesis of bioactive dithiobis(phenylene)bis(benzylideneimine) via sequential oxidative coupling-condensation reactions in one pot from aminothiophenol and benzaldehyde

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Abstract: Bovine serum albumin (BSA) has been explored for aerobic oxidative coupling of thiols (aromatic, heterocyclic as well as aliphatic) “on water” towards formation of disulfides (S-S) without using any metal/non-metal complexes, bases and additives, which renders the process environmentally benign and economically attractive with good recyclability (upto four cycles). The developed green protocol was further extended for synthesis of diallyldisulfide (DADS), an important constituent of natural occurring allicin. Among various synthesized disulfides, bis(2-aminophenyl)disulfide, obtained by oxidative coupling of 2-aminothiophenol in BSA, was further utilized for condensation with benzaldehyde in the same pot thus enabling easy access to bioactive dithiobis(phenylene)bis(benzyldeneimine). This is the first example of BSA catalyzed sequential (oxidation/condensation) reaction where one S-S and two C-N bonds are formed solely "on water."

Keywords: Bovine Serum Albumin, Disulfide, Oxidative Coupling-Condensation reactions, Thiophenol, Waste-free Protocol, Water.

1. Introduction

The organic synthesis with minimal environmental impact has become focus of chemists and the use of natural catalysts [1-6] (viz. proteins, enzymes, whole cell etc.) have steadily ascended owing to inherited environmental and ecological benefits. In the same vein, the use of water to facilitate eco-sustainable chemical reactions is also gaining momentum because either its use as a solvent or its release as a by-product, will clearly have the least impact on the environment [7-9]. Furthermore, there is representative "on water" [10-16] reactions termed by Sharpless and co-workers [10] where conducting the reaction "on water" can be substantially beneficial because of its high heat capacity, increase in reaction rate, selectivity and an improved safety profile with ease of operation due to the insolubility of both reactants and products in aqueous conditions. In this context, numerous organic transformations including oxidation of aldehyde into carboxylic acid "on water" using molecular oxygen as an environmentally benign and sustainable oxidant have surfaced [11]. Nevertheless, aerobic oxidation reactions promoted by biocatalysts under neutral reaction conditions are rare and lack generality "on water."

Among various oxidation reactions, the selective oxidative coupling of thiols (-SH) to corresponding disulfides (S-S bond) under mild reaction conditions is critical from biological and chemical viewpoints [17-26]. The formation of disulfide bond is also a matter of interest for the industrial production of some agrochemicals and pharmaceuticals [27-37] like pyritinol [33], DADS (a major decomposition product of allicin found in *Allium sativa*) [36] and dithiobis(phenylene)bis(benzyldeneimine), an antimicrobial agent against human pathogens [34,35] (Fig.1).

Conventionally, a large number of oxidative coupling protocols [38-55] including ball-milling [38], Fe(BTC) [40], diaryltelluride [43], gold nanoparticles [46] and hypervalent iodine(III) [47] have been documented for the synthesis of disulfides [52-55]. However, most of these approaches suffer from inadequacies such as low selectivity due to over oxidation [42] of final product into sulphoxides/sulphones, expensive and toxic nature of catalysts with poor recovery and finally laborious work up procedures with generation of a lot of waste materials. Recently, Wu and co-workers [51] have reported an effective and recyclable photocatalytic method for selective conversion of thiols into disulphides with high turnover number (TON) but visible-light irradiation of quantum dots is necessary. Against this milieu, it is a surprise that only few biocatalysts [56-58] including horseradish peroxidase or mushroom tyrosinase [56], baker's yeast [57] and enzyme laccases [58] have been known for aerobic oxidative coupling of thiols into disulfides but these reactions occurs in aqueous-organic medium along with maintenance of pH conditions [56] and use of ABTS as radical initiator [58]. So there is a strong need to develop such a biocatalytic system for oxidation of thiols to disulfides which comprises all, i.e. inexpensive biocatalyst, aerobic oxygen and water as a sole reaction medium besides recyclability of the biocatalyst (Fig. 2).

In this context, BSA [59-68] (also known as "Fraction V") an ubiquitous and inexpensive, non-enzymatic transport protein derived from cows, occupies a unique position due to its versatility to catalyze an array of chemical reactions [59-68, 69-71] including the asymmetric thia-Michael [72] reaction. It is one of the most studied proteins that have a strong affinity to bind organic molecules by reversible non-covalent complexation in its hydrophobic pockets thus providing an environment for a number of organic transformations including reduction [60], Knoevenagel condensation [61] and Gewald reaction [62]. Based upon our ongoing interest on BSA catalyzed Aldol/Knoevenagel-Doebner condensation [69], multicomponent Biginelli reaction [70] besides oxidation of tertiary amine to N-oxide at pH 9

by Colonna et al [59], we ventured to explore BSA as a biocatalyst for waste-free aerobic oxidation of thiols to disulfides "on water" which received considerable interest for various chemical transformations [10-16] as water is a cheap, nontoxic solvent as well as it increases the reactivity or selectivity of the reaction, often difficult to attain in organic solvents.

Among various disulfides (Fig. 1), bis(2-aminophenyl) disulfide [73-81] is particularly known to participate towards formation of bis(2-aminophenyl)disulfidediimines, a Schiff bases ligand [73] (Fig. 3) which possess sound antibacterial activity [74] and are also used as basic scaffold in various chemical transformations [75-78]. Despite promising biological profiles, synthesis of these Schiff bases requires two steps involving metal/base [38,39,42,47] catalyzed oxidation of 2-aminothiophenol into corresponding bis(2-aminophenyl)disulfide which upon isolation and purification followed by condensation with benzaldehyde in anhydrous [73] ethanol affords the desired product bis(2-aminophenyl)disulfidediimines (Fig. 3) having one S-S and two C-N bonds. Against this drawback, creation of multiple bonds [82-84] in one pot without changing the reaction condition and isolating the intermediate helps in minimizing waste, time and energy which are most important aspects of green chemistry and generally accomplished by tandem [85-88] (consecutive/sequential) reactions. It is worth mentioning that 2-aminothiophenol which is a versatile synthon [73-81], on one side undergoes self-oxidative coupling into product bis(2-aminophenyl)disulfide [47] while on the other side it undergoes condensation-cyclisation with benzaldehyde leading to benzothiazole [89-91]. Hence it would be very challenging if aerobic oxidative coupling-condensation between 2-aminothiophenol and benzaldehyde undergoes in a tandem manner in one pot towards formation of bis(2-aminophenyl)disulfidediimines (Schiff base) instead of benzothiazole on water (Fig. 3). Further, formation of these Schiff bases [73] on water under neutral conditions would be an interesting preposition as generally condensation between amine and carbonyl rather

demands water removal [92-95] by azeotropic distillation [90] or use of lewis acid [94] catalysts/dehydrating agents [95]. Hence the BSA possessing a rich diversity of surface amino acids would seems effective towards formation of Schiff base as well as disulphides ligands having miscellaneous medicinal and physicochemical profiles [33,35-36,61-62,73-74, 99-108].

In this context, we disclose for the first time, a neutral and waste free protocol for direct aerobic oxidative coupling of thiols into disulfides "on water" utilizing BSA as a biocatalyst which is successfully extended for the synthesis of bis(2-aminophenyl)disulphidediimines via sequential (oxidative coupling-condensation) reaction in one pot (Fig. 2 and 3) without using any metal oxidant/catalyst, base or organic solvents. The recyclability of the biocatalyst is also noteworthy which emphatically demonstrates the benign nature of the protocol.

2. Experimental Section

2.1 Materials:-

All the substrates/chemicals were obtained from commercial sources (Sigma Aldrich and Alfa Aesar). The albumins (Bovine, Pig, Sheep, Chicken, Rabbit) and Lipases (PPL= *Porcine pancreas lipase*, CAL-B= *Candida antarctica lipase*, CCL= *Candida cylindracea*; MJL= *Mucor javanicus*, CRL= *Candida rugosa lipase*; TLL= *Thermomyces lanugenosus lipase*; PCL= *Pseudomonas cepacia lipase*) used in this work have been purchased from Sigma Aldrich. L-Arginine, Lysine and other amino acids were purchased from Sigma Aldrich and Himedia. Distilled water was used in all reactions. The solvents used for isolation/purification of compounds were obtained from commercial sources (Merck) and were used without further purification.

149 **2.2 Analytical method:**

150 The GC-MS analysis was carried out on Shimadzu MS-QP-2010 system equipped with a
151 BP-20 capillary column (SGE international). ^{1}H (300 MHz and 400 MHz) and ^{13}C (75.4 MHz
152 and 100 MHz) NMR spectra were recorded on Bruker Avance-300 and 400 spectrometer.
153 The following abbreviations were used to designate chemical shift multiplicities: s = singlet,
154 br = broad, d = doublet, t = triplet, q = quartet, m = multiplet. The ^{13}C NMR spectras are
155 proton decoupled. Melting points were obtained manually by capillary methods and are
156 uncorrected. Column chromatography was done on silica gel (60-100 mesh size). Thin layer
157 chromatography (TLC) was performed on silica TLC plates and compounds were visualized
158 in iodine and under UV lamp.

159 **2.3 General Procedure**

160 **2.3.1 General procedure for the synthesis of disulfides (1b-27b):**

161 Thiophenol (0.25 mmol), BSA (50 mg) and deionised water (600 μL) were stirred at room
162 temperature for 12h or till completion of reaction (monitored by TLC). Then water (2 mL)
163 was added to reaction mixture and extracted with ethyl acetate (3mL X 2), organic part dried
164 over Na_2SO_4, filtered and then concentrated under rotary evaporator and chromatographed
165 over silica gel by hexane/ethyl acetate (99:1). The obtained disulfides (1b-27b) were
166 characterized and confirmed by comparing the ^{1}H NMR and ^{13}C NMR data with those
167 reported in the literature [38-51,109-117].

168 **2.3.2 General procedure for bis(2-aminophenyl)disulfidediimine Schiff bases (28b-30b):**
169 2-Aminothiophenol (0.25 mmol), BSA (50 mg) and deionised water (600 μL) were stirred at
170 room temperature for 18h to obtain the desired intermediate bis(2-aminophenyl)disulfide (4b)
171 followed by addition of benzaldehyde (1.1 equiv.) and BSA (50 mg) in the same pot. The
172 reaction mixture was further stirred at room temperature for 12h (monitored on TLC). After

the completion of reaction, water (2 mL) was added to the reaction mixture and extracted with ethyl acetate (3ml X 2), organic part dried over Na_2SO_4, filtered and concentrated under rotary evaporator and recrystallized with methanol to obtained pure product. The obtained products (28b-30b) were characterized and confirmed by comparing the ^1H NMR and ^{13}C NMR data with those reported in the literature [75].

2.4 (^1H & ^{13}C) NMR values of isolated compound (1b-30b):

2.4.1. Diphenyldisulfide (1b) [45]

White solid, mp: 58-61°C (lit [45] 61-62°C), ^1H NMR ($CDCl_3$, 400 MHz): δ 7.54-7.52 (4H, m), 7.35-7.24 (6H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 137.0, 129.0, 127.6, 127.2.

2.4.2. Bis(2-naphthalenyl)disulfide (2b) [45]

White solid, mp: 133-136°C (lit [42] 136-137°C), ^1H NMR ($CDCl_3$, 300 MHz): δ 8.03 (2H, s), 7.83-7.75 (6H, m), 7.68 (2H, d, J = 8.63 Hz), 7.52 (4H, m); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 134.7, 133.9, 132.9, 129.4, 128.2, 127.9, 127.2, 127.0, 126.7, 126.1.

2.4.3. Bis(4-methoxyphenyl)disulfide(3b) [45]

White solid, mp: 33-36°C (lit [44] 34-35°C), ^1H NMR ($CDCl_3$, 300 MHz): δ 7.44 (4H, d, J = 8.77 Hz), 6.88 (4H, d, J = 8.75 Hz), 3.83 (6H, s); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 160.3, 133.1, 128.8, 115.0, 55.8.

2.4.4. Bis(2-aminophenyl)disulfide (4b) [45]

Yellow solid, mp: 90-94°C (lit [44] 93-94°C), ^1H NMR ($CDCl_3$, 300 MHz): δ 7.21-7.16 (4H, m), 6.74 (2H, d, J = 8.48 Hz), 6.64 (2H, d, J = 8.48 Hz), 4.35 (4H, s); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 149.1, 137.2, 132.0, 119.2, 118.7, 115.7.

2.4.5. Bis(4-hydroxyphenyl)disulfide (5b) [45]

Yellow solid, mp: 152 –154^{0}C (lit [44] 150–151^{0}C), ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.83 (2H, s), 7.29-7.27 (4H, m), 6.78-6.75 (4H, m); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.8, 133.5, 125.6, 116.8.

2.4.6. Bis(3,4-dimethoxyphenyl)disulfide (6b) [109]

White solid, mp: 90-92°C (lit [109] 94°C), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.09-7.04 (4H, m), 6.82 (2H, d, J= 8Hz), 3.89 (6H, s), 3.85(6H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.3, 128.7, 124.0, 114.2, 111.4, 56.0.

2.4. 7. Bis(2-methylphenyl)disulfide (7b) [45]

White solid, mp: 39-40^{0}C (lit. [48] 38-39°C), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42 (4H, d, J = 8.14 Hz), 7.14 (4H, d, J = 8.02 Hz), 2.35 (6H, s); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 137.8, 134.8, 130.2, 129.0, 21.5.

2.4.8. Bis(2-methoxyphenyl)disulfide (8b) [41]

White solid, mp: 119–120^{0}C (lit [41] 120°C), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.64-7.61 (2H, m), 7.26-7.22 (2H, m), 6.99-6.95 (2H, m), 6.95–6.89 (2H, m) 3.93 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz,): δ 156.5, 127.7, 127.5, 124.5, 121.3, 110.4, 55.9.

2.4.9. Bis(4-methoxybenzyl)disulfide (9b) [110]

White solid, mp: 100-102°C (lit [110] 100°C) ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.20-7.18 (4H, m), 6.90-6.86 (4H, m), 3.83 (3H, s) 3.63 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.0, 130.5, 129.4, 113.9, 55.29, 42.81.

2.4.10. Bis(4-methylphenyl)disulfide(10b) [45]

White solid, mp: 82-84°C (lit [44] 80-82°C), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.43 (4H, d, J = 8.19 Hz), 7.15 (4H, d, J = 7.97 Hz), 2.35 (6H, s); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 137.9, 134.3, 130.2, 129.0. 21.5.

2.4.11. Bis(3-chlorophenyl)disulfide (11b) [44]

White solid, mp: 70-72°C (lit [44] 70-72°C),^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.51 (2H, s), 7.39-7.33 (2H, m), 7.26-7.20 (4H, m); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 138.8, 135.6, 130.6, 128.0, 127.4, 125.8.

2.4.12. Bis(2-nitrophenyl)disulfide, (12b) [111]

Yellow solid, mp: 192°C (lit [112] 192°C), ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.34-8.32 (2H, m), 7.82-7.80 (2H, d, J=8 Hz), 7.7-7.66 (2H, m), 7.51-7.47(2H, m); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 144.4, 133.8, 132.5, 126.3, 125.7, 125.1.

2.4.13. Bis(4-fluorophenyl)disulfide (13b) [45]

Yellowish oil,^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.49-7.46 (4 H, m), 7.06-7.01 (4H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 163.8, 161.4, 131.4, 131.3, 116.4, 116.2.

2.4.14. Bis(4-bromophenyl)disulfide(14b) [44]

White solid, mp: 92-96°C (lit [44] 94-95°C), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45-7.42 (4H, m), 7.37-7.33 (4H, m); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 135.1, 132.6, 129.8, 121.9.

2.4.15. Bis(2-bromophenyl)disulfide (15b) [113]

White solid, mp: 95-97°C (lit [113] 97°C), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.57-7.55 (4H, m), 7.31-7.27 (2H, m), 7.13-7.08 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.2, 132, 128.2, 128.0, 127.0, 121.2.

2.4.16. Bis(4-chlorophenyl)disulfide (16b) [44]

White solid, mp: 62-65°C (lit [44] 65-66°C), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45 (4H, d, J = 8.65 Hz), 7.31 (4H, d, J = 8.65 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz); δ 135.6, 134.1, 129.8, 129.7.

2.4.17. Bis(phenylmethyl)disulfide(17b) [45]

White solid, mp: 57-60°C (lit [44] 58-59 °C), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39-7.26 (10H, m), 3.64 (4H, s); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 137.8, 129.8, 128.9, 127.8, 43.7.

2.4.18. Dicyclohexyldisulfide (18b) [44]

Colorless oil, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.73-2.64 (2H, m), 2.08-2.04 (4H, m), 1.80-1.79 (4H, m), 1.64-1.61 (2H, m), 1.35-1.26 (10H, m), ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 50.4, 41.3, 33.2, 26.5, 26.1.

2.4.19. L-Cystine(19b) [49]

White solid, mp: 238-239^{0}C (lit [43] 240-242^{0}C) ^{1}H NMR (D_2O+4%NaOD, 400 MHz): δ 3.59-3.57 (2H, m), 3.14-3.09 (2H, m), 2.94-2.88 (2H, m); ^{13}C NMR (D_2O+4%NaOD, 100 MHz): δ 180.5, 54.8, 43.3.

2.4.20. Bis(2-pyridinyl)disulphide (20b) [45]

White solid, mp: 53-56°C (lit [45] 53-54°C), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.50 (2H, m), 7.63 (4H, m), 7.16-7.13 (1H, m); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 158.9, 149.5, 137.4, 121.1, 120.0

2.4.21. Bis(pyrimidin-2-yl)disulphide (21b) [45]

Yellow solid, mp: 144-145°C (lit [45] 143-145°C), ^{1}H NMR (DMSO-d_6 + $CDCl_3$, 400 MHz): δ 8.47-8.46 (4H, m), 7.08-7.06 (2H, m); ^{13}C NMR (DMSO-d_6 + $CDCl_3$, 100 MHz): δ 173.8, 163.0, 123.0

2.4.22. Bis(2-pyridinoxydyl)disulfide (22b) [114]

Yellow solid, mp: 202-204°C (lit [114] 205°C), ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.48 (2H, s), 7.80.-7.3 (6H, m); ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ 151.0, 139.5, 139.0, 127.9, 124.4, 122.7.

2.4.23. Bis(thiazol-2-yl)disulfide (23b) [115]

White solid, mp: 79°C (lit [115] 79-80°C), ^{1}H NMR ($CDCl_3$, 300 MHz):δ 7.80 (2H, d) 7.42 (2H, d); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 176.9, 144.3, 122.4.

2.4.24. Bis(benzo[d]thiazol-2-yl)disulfide (24b) [45]

White solid, mp: 176-177^{0}C (lit [45] 176-178^{0}C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.98-7.96 (2H, d, J= 8Hz), 7.81-7.79 (2H, d, J= 8Hz), 7.51-7.47 (2H, t, J= 8Hz), 7.40-7.36 (2H, t, J= 8Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.8, 154.6, 136.2, 126.6, 125.3, 122.8, 121.3.

2.4.25. Bis(benzo[d]oxazol-2-yl)disulfide (25b) [45]

White solid, mp: 94-96°C (lit [45] 92-94°C), ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.77-7.75 (4H, m), 7.44-7.41 (4H, m); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 159.8, 152.0, 141.2, 126.0, 125.3, 119.7, 111.0

2.4.26. 5,5'-disulfanediylbis(1,3,4-thiadiazol-2-amine) (26b) [116]

White solid, mp: 238-241^{0}C (lit [116] 239^{0}C), ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.74 (2H, s); ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ 173.0, 149.5.

2.4.27. 1,2-diallyldisulphide (27b) [117]

Yellow oil, ^{1}H NMR ($CDCl_3$, 400MHz): δ 5.92-5.75 (2H, m) 5.24-5.10 (4H, m), 3.16-3.14 (4H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 134.39, 118.5, 42.4.

Spectral data of bis(2-aminophenyl)disulfidediimine Schiff bases (28b-30b):

2.4.28. 2,2´-disulfanediylbis(N-benzylideneaniline) (28b) [73]

Yellow solid, mp: 140^{0}C (lit [73] 140°C), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.54 (s, 2H), 8.03-8.00 (m, 4H), 7.71-7.69 (m, 2H), 7.53-7.49 (m, 6H), 7.28-7.16 (m, 4H), 7.09-7.07 (m, 2H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 160.0, 148.9, 136.0, 132.1, 131.7, 129.1, 128.8, 126.9, 126.0 , 117.2.

2.4.29. 2,2´-disulfanediylbis(N-(4-methoxybenzylidene)aniline) (29b)[73]

Yellow solid, mp: 140^{0}C (lit [73] 139 -140°C), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.44 (^{1}H, s), 7.97-7.95 (4H, m), 7.66 (1H, m),7.28–7.02 (12H, m), 3.91 (6H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 162.5, 159.2, 149.2, 131.9, 130.9, 129.2, 126.8, 126.4, 125.8, 117.1, 114.2, 55.3.

2.4.30. 2,2'-disulfanediylbis(N-(pyridin-2-ylmethylene)aniline) (30b)[74]

Yellow solid, mp: 141^{0}C (lit [74**]** 139-140°C),^{1}H NMR (DMSO-d_6, 400 MHz,) δ 8.72 (s, 2H), 8.30 – 8.21 (m, 2H), 7.62 – 7.50 (m, 5H), 7.44 – 7.23 (m, 9H); ^{13}C NMR (DMSO-d_6, 100 MHz) : 160.4, 154.5, 149.7, 147.8, 136.8, 132.7, 127.9, 127.1, 126.0, 125.5, 122.2, 117.3.

3.0 Results and Discussion

Inspired by the outcomes of recent reports [69-72] on the decisive role of BSA catalyzed (i) enone formation in ionic liquid [69] and (ii) thia-Michael addition to chalcone in water [72],

we planned to carry out the BSA catalyzed one-pot multicomponent reaction among benzaldehyde, acetone and thiophenol in water via enone formation followed by thia-Michael addition in the same pot (see supporting information). Unpredictably, we got some competing side product (58% on GC-MS basis) which upon isolation and characterization by NMR, GC-MS and HPLC comparison with standard [44], was confirmed to be diphenyldisulfide (see S.I.). This result encouraged us to stir a mixture of thiophenol (0.25 mmol) (1a) and bovine serum albumin [69,70] (50 mg, 0.00075 mmol, molecular weight = 66000 [59]) "on water" [10] under aerobic [11] conditions for 12h, thereby providing diphenyldisulfide (1b) in 85% yield (Entry 1, Table 1, GC-MS basis). Delighted by this serendipitous BSA protein catalysis of **1a**, we screened different commercially available serum proteins viz. rabbit, sheep, pig, chicken, egg albumin to improve the yield of **1b**, however, in all cases **1b** was obtained in poor yield (Entry 2-6, Table 1).

During the screening of different serum proteins (Entries 1-6, Table 1), it was realized that conversion of thiol (1a) into disulfide (1b) with protein BSA (Entry 1, Table 1) was found optimum which may be due to the rich varieties of surface amino acids [61,62] present in it. Recently, *Laccase* enzyme [58] has also been utilized for oxidative coupling of thiols into disulfides, therefore, we further planned to employ some well exploited and commercially available enzymes [71] (Entries 7-12, Table 1) like CAL-B (*Candida antarctica lipase*), CRL (*Candida rugosa lipase*), MJL (*Mucor javanicus lipase*), PCL (*Pseudomonas cepacia lipase*), and CCL(*Candida cylindracea lipase*) for oxidation of thiol (1a) into disulfide (1b) on water but none of these lipases showed any significant product (1b) formation (Entries 7-12, Table 1) in comparison to protein BSA (Entry 1, Table 1).

Next to improve the yield of **1b** beyond 85% (Entry 1, Table 1), variations in time, effect of temperature, solvents and amount of BSA were studied (Entries 2-15,Table 2). During variations in time (Entries 1-3, Table 2), it was found that 12 h (Entry 1) was

sufficient for the oxidative coupling of **1a** while increase (24 h) or decrease (6 h) in reaction time did not show any improvement in yield of **1b** (Entries 2-3, Table 2). Further, variations in reaction temperature (Entries 4-5,Table 2) and amount of BSA (Entries 6-7) did not show any influence on the improvement of yield of **1b**. Thereafter, the change in reaction medium from water to organic solvents (Entries 8-12,Table 2) were taken into account but no significant improvement in the yield of **1b** was observed. Further to validate our perception regarding the crucial role of BSA, dissolved oxygen in water or aerobic oxygen for oxidation of **1a** into **1b**, we performed a set of three reactions i) aerobic oxidation of **1a** without BSA in water (Entry 13, Table 2), ii) oxidation of **1a** with BSA and water under N_2 atmosphere (to ward off oxygen) (Entry 14, Table 2) and iii) oxidation of **1a** with BSA in degassed water (to ward off oxygen) under N_2 atmosphere (Entry 15) wherein yield of **1b** was obtained in 0%, 37% and traces amount respectively (on the basis of GC-MS analysis). These findings clearly highlighted the crucial role of BSA and aerial oxygen for facile oxidative coupling of **1a** into **1b**. Among various proposed reaction pathway for oxidative coupling of thiols, a thiyl radical [58] pathway is generally known for S-S bond formation. To ascertain whether the aerobic oxidation of **1a** follows a free radical pathway or not, an experiment with 10 mol% BHT (a free radical quencher) (Entry 16, Table 2) as well as oxidation of **1a** in dark (Entry 17, Table 2) were conducted and in both cases **1b** was obtained in good yield, hence, ruling out the possibility of free radical pathway.

After optimal reaction conditions in hand for oxidative coupling of **1a**, we examined the scope of different substituted thiols to form the disulfides (1b-18b, Table 3) including synthesis of a physiologically important cystine (19b, Table 3). As shown in Table 3 (1b-19b), a wide range of thiophenols with electron-donating (2b-10b, Table 3) and electron-withdrawing (11b-16b) substituents have provided good to excellent yields (upto 97%) of disulfides [96-98] wherein some of disulfides possess cytotoxic [97], leishmanial [98] and

antibacterial activities [74]. Delightedly, amino or hydroxy substituted thiophenol (4a and 5a, Table 3) also successfully underwent oxidative coupling to form corresponding disulfide (4b and 5b, Table 3) in good yield (upto 85%) whereas previous protocols [44-46] documented the formation of the same disulphides (4b and 5b) in poor yield due to additional interaction of -NH_2 or -OH proton verses -SH proton of thiophenol with catalysts/reagents [44]. Interestingly, BSA possesses rich variety of amino acids which will interact efficiently with -SH of thiophenol in water whereas strong hydrogen bonding ability of –OH and –NH_2 in water may attenuate their interaction with biocatalyst (BSA) thereby permitting smooth oxidative coupling of thiophenols (4a and 5a) into disulfides (4b and 5b, Table 3).

Apart from aromatic disulphides (1b-17b, Table 3), many heteroaromatic disulfides (20b-26b, Table 4) are privileged scaffolds of some medicinally active compounds [27-37], thus high therapeutic profile and their recent biocatalyzed synthesis by U. Beifuss et al [58] propelled us to target synthesis of these heteroaromatic disulfides (20b-26b, Table 4) in our neutral catalytic system (BSA/water) devoid of any additives or use of organic solvent. We successfully obtained six membered heterocyclic disulfides with excellent yield (upto 90%) having pyridine and pyrimidine ring (20b-26b, Table 4) which are documented for production of a dendritic cell-based vaccine for HIV [35] as well as a drug for neuropsychological disorders [33]. Similarly, five membered heterocyclic disulfides (23b-26b, Table 4) were also obtained in moderate yield (upto 65%).

In order to further extend the application of developed protocol for oxidation of aliphatic thiols, the synthesis of bioactive [36, 99-101] diallyl-disulfide (DADS) (27b), a well-known secondary metabolite of garlic (*Allium sativa*) was targeted. Thus, BSA catalyzed oxidation of allylthiol (Scheme1) on water provided diallyldisulfide (DADS) in 78% yield (isolated) under environmentally benign aerobic condition.

Mechanistically, we hypothesized that acidic and basic amino acid functionalities in the side chain of BSA [64-67] might be responsible for oxidative coupling of thiophenols into disulfides in aqueous condition. In fact, structurally the BSA, a non-redox carrier protein, contains side chains of acidic (Asp, Glu) [64] and basic (Arg, Lys, His)[64] amino acids which could act as an ambiphile [102-104] in water. In this view, disulfides formation might be driven through transition state {A} (Scheme 2) wherein sulfur of thiophenol interacts with acidic hydrogen of surface amino acid side chains of BSA analogues to carbonyl oxygen activation by oxyanion hole [105-108] in enzyme catalysis [59-68, 105-107] or amino acid catalysis [97] thereby imparting the partial positive character to sulfur of thiophenol. On the other side the basic amino side chains [59-71] of BSA as reported previously [70,102] by Zhao et al. [62] or heterocyclic amino acids like histidine [62,63,105] present in BSA could somewhat behave like base thereby their interaction with -SH proton (of another molecule of thiophenol) impart nucleophilic character to sulfur. Overall this ambiphilic [49,102-104] (electrophilic and nucleophilic) activation triggers interaction of nucleophilic (δ-) and electrophilic (δ+) sulfur, which was further assisted by aerobic oxygen and water through hydrogen bonding (HB) leading to diphenyldisulfide with removal of water as the only by product.

Furthermore to investigate an insight in the crucial role of amino acids (acidic or basic) side chain present in BSA, we carried out oxidative coupling of thiophenol (1a) with commercially available acidic (Glutamic acid, Aspartic acid) and basic (Arginine and Lysine) amino acids in water (see S.I.). To our delight, arginine provided maximum yield (52%) while glutamic acid provided 37% yield of **1b** (see S.I.) which overall supports the synergetic role of both acidic and basic amino acid functionalities present in BSA which might be responsible for high yield of disulfide (85%, 1b, Table 3) under above catalytic system (i.e.

BSA-water-air). Further, reduction in yield of disulfide (1b) with acylated and denatured [70] BSA also confirms our mechanistic postulate (see S.I.).

Having established substrates scope and plausible mechanism, we proceeded to assess the recyclability of the biocatalytic system for oxidation of 4-methoxy thiophenol (3a) into bis(4-methoxyphenyl) disulfide (3b, Table 3) where the recovered catalyst retained activity upto four cycles, thereafter yield of **3b** decreased below 90% (Fig. 4) due to coagulation of BSA. The E-factor (see S.I.) of our biocatalytic system were also found to be low for **3b** which further confirms environmental compatibility of this biocatalytic protocol according to green chemistry metrics.

To probe the practical applicability of our catalytic system (Table 3) for disulfides synthesis, preparative scale oxidative coupling reaction of 4-methoxythiophenol (3a, 1g, 7.1 mmol) using BSA (200 mg) in water (2 mL), at room temperature (16 h) formed corresponding disulphide, bis(4-methoxyphenyl)disulfide (3b, Table 3) in excellent yield upto 92% (see S.I.) precluding any metal/non-metal complexes, bases and additives as shown in Table 5 which clearly demonstrates its applicability in comparison to reported protocols [38-47, 56-58,118-133].

After the successful oxidation of thiols (aromatic, heterocyclic, aliphatic) into corresponding disulfides (1b-27b, Table 3, 4 and scheme 1) and establishing the mechanistic study (Scheme 2), we envisioned that bis(2-amino diphenyl)disulfide, an amino functionalised disulfide (4b, Table 3), can further be reacted with benzaldehyde to form bis(aminophenyl)disulfidediimine which possesses good antimicrobial activity against human pathogens [73] and also documented as an important ligand in synthetic chemistry [74] besides acting as a basic scaffold for various organic synthesis [75-79]. Thus, in order to examine the application of our developed biocatalytic green protocol for tandem [85-88] oxidative coupling as well as condensation reactions between aminothiophenol and benzaldehyde, we particularly added

benzaldehyde in parts (to avoid benzothiazole formation) [89-91] to a well stirred mixture of 2-aminothiophenol and BSA in water but unfortunately, the desired bis(2-aminophenyl)disulfidediimine was formed only in trace amount with many side-products (Scheme 3). So in order to elude the multiple product formation due to competitive reactions (Scheme 4), we next followed tandem sequential [88] reaction wherein initial formation of diiminedisulfide (4b) was achieved in 18 h. After that benzaldehyde (1.1eq) was added in the same pot and further stirred for 12h at r.t. which successfully furnished desired product (28b) in 77% yield (Scheme 3) via oxidative coupling and condensation reaction in one pot. Similarly, disulfidediimines (**29b-30b)** were also obtained with good yields via tandem-sequential oxidative coupling-condensation in the same pot under aerobic aqueous conditions (Scheme 4) while reported methods [73-74] involve multi steps and demand anhydrous conditions. Thus among the richness of various organic transformations catalyzed by BSA [134], our method may be employed as a mild approach for disulfides synthesis along with extended approach towards Schiff bases

4.0 Conclusions

In summary, this is the first report on bovine serum albumin (BSA) catalyzed oxidative coupling of a series of thiols (aromatic, aliphatic and heterocyclic) into disulfides (S-S bonds) "on water" under neutral conditions whose significant features are: (i) metal and base-free protocol, (ii) air as a green oxidant, (iii) water as a green solvent, (iv) mild reaction conditions and easy isolation of the products, (v) waste free protocol and (vi) good recyclability with high atom economy and low E factor. More importantly, the versatility of protocol finds application for synthesis of 4,5-dithia-1,7-octadiene (diallyldisulphide commonly known as DADS), a biologically important secondary metabolite of garlic (*Allium sativa*). Furthermore, the utility of the methodology for an efficient construction of

biologically active bis(aminophenyl)disulfidediimine is also demonstrated where BSA catalyzed one S-S and two C-N bonds are formed for the first time solely "on water" in one pot via sequential oxidative coupling of 2-aminothiophenol into intermediate bis(2-aminophenyl)disulfide followed by condensation with benzaldehyde without isolation of intermediate, thus the developed protocol is of great value from the green chemistry perspective and organic synthesis due to inexpensive nature of BSA, air as a green oxidant and water as a solvent. Work is underway toward the further development of relevant reactions.

Acknowledgements

Authors are gratefully acknowledge the Director (s) of C.S.I.R.-C.D.R.I., Lucknow and C.S.I.R.-I.H.B.T., Palampur for their kind cooperation and encouragement. Some part of work carried out at I.H.B.T. (3537). The authors are also grateful to SAIF division of CDRI and Mr. S Kumar/Mrs. V. Pathania of I.H.B.T. for providing spectral data. CDRI Communication No. 36/2015.

†Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x

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Figure Captions

Figure 1: Biological active scaffolds containing S-S bond

Figure 2: BSA catalysed first oxidative coupling of thiols into disulfides (S-S bond) under neutral condition

Figure 3: Synthesis of Schiff bases with one S-S and two C-N bonds via oxidative coupling-condensation reactions in one pot

Figure 4: Recyclability of BSA in developed protocol

671 **Table 1: Screening of different serum proteins and enzymes for oxidation of thiophenol**
672 **(1a) into disulfide (1b)[a]**

673 **[a] Experimental conditions: 0.25 mmol 1a, 600 µL H_2O for 12 hr; [b] yield on the basis**
674 **of GC-MS; (BSA=Bovine serum albumin; CAL-B= *Candida antarctica lipase-B*; CRL=**
675 ***Candida rugosa lipase*; MJL=*Mucor javanicus lipase;* TLL= *Thermomyces lanugenosus***
676 ***lipase*; PCL= *Pseudomonas cepacia lipase;* CCL=*Candida cylindracea lipase*.)**

677 **Table 2: Optimization of BSA catalyzed disulfide synthesis [a]**

678 **[a] Experimental conditions: 0.25 mmol 1a, 600 µL solvent; BSA= Bovine serum albumin**
679 **50 mg; [b] Yield on the basis of GC-MS; [c] without BSA; [d] under N_2 atmosphere; [e] in**
680 **the presence of BHT (Butylatedhydroxytoluene); [f] reaction in dark**

681 **Table 3: Substrate scope for oxidative coupling of aromatic thiols into disulfides in**
682 **BSA-water-oxygen catalytic system[a]**

683 **[a] Reaction conditions: Thiol 0.25 mmol, BSA 50 mg, water 600 µL, rt, reaction time**
684 **(12-48 h); [b] Isolated yield; [c] yield on GC-MS basis**

685 **Table 4: Substrate scope for oxidative coupling of heterocyclic thiols into disulfides in**
686 **BSA-water-oxygen catalytic system[a]**

687 **[a] Experimental conditions: Thiol 0.25 mmol, BSA= 50 mg, Water= 600 µL; [b]**
688 **Isolated yield**

689 **Table 5: Substrate scope for oxidative coupling of heterocyclic thiols into disulfides in**
690 **BSA-water-oxygen catalytic system[a]**

691

692 **Scheme 1: Synthesis of diallyldisulfide (DADS)**

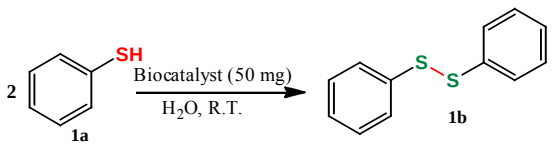
693 **Scheme 2: Plausible mechanism for disulfide formation**

694 **Scheme 3: Formation of bis(aminophenyl)disulfidediimine in tandem sequential manner**

695

Tables

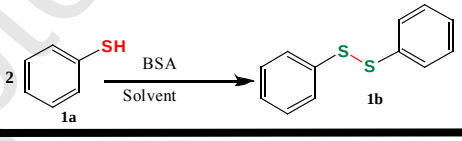
Table 1: Screening of different serum proteins and enzymes for oxidation of thiophenol (1a) into disulfide (1b)[a]



S. No.	Biocatalyst	% Yield[b]	S. No.	Biocatalyst	% Yield[b]
1	BSA	85%	**7**	CAL-B	nd
2	Rabbit serum albumin	12%	**8**	CRL	2
3	Sheep serum albumin	16%	**9**	MJL	nd
4	Pig serum albumin	13%	**10**	TLL	nd
5	Egg serum albumin	11%	**11**	PCL	nd
6	Chicken albumin	10%	**12**	CCL	nd

[a] Experimental conditions: 0.25 mmol **1a**, 600 µL H_2O for 12h; [b] yield on the basis of GC-MS; (BSA=Bovine serum albumin; CAL-B= *Candida antarctica lipase-B*; CRL= *Candida rugosa lipase*; MJL=*Mucor javanicus lipase*; TLL= *Thermomyces lanugenosus lipase*; PCL= *Pseudomonas cepacia lipase;* CCL=*Candida cylindracea lipase*.)

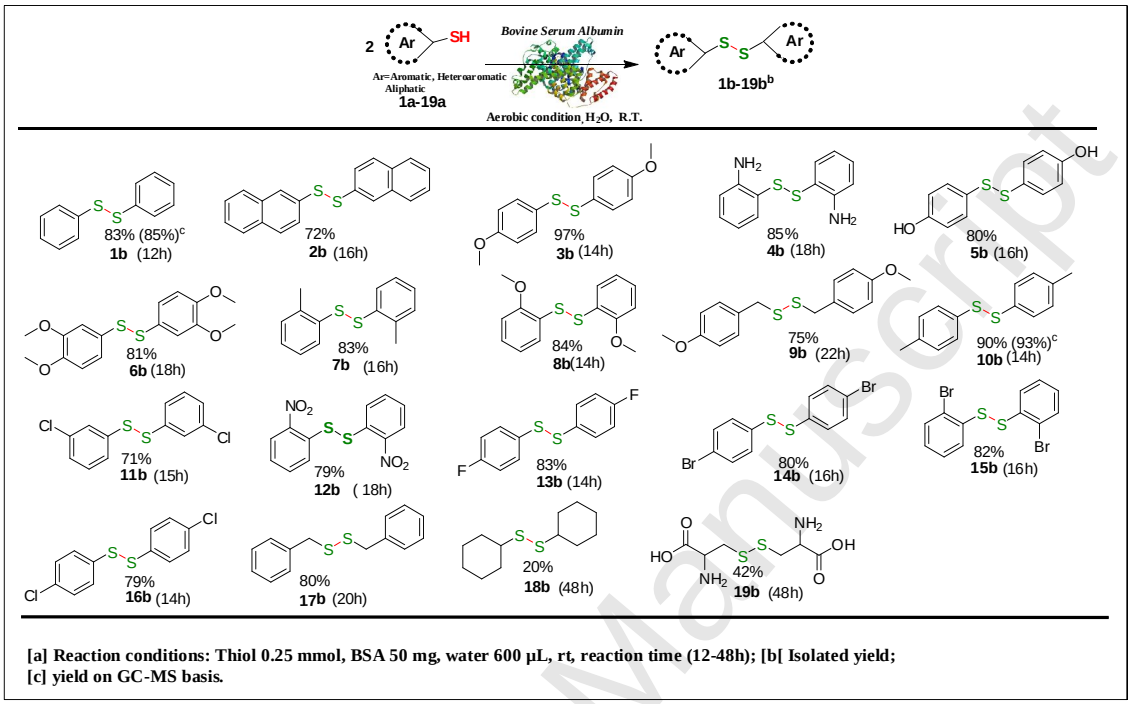
Table 2: Optimization of BSA catalyzed disulfide synthesis[a]



S. No.	Solvent	BSA (mg)	Time (h)	Temperature	% Yield[b]	S. No.	Solvent	BSA (mg)	Time (h)	Temperature	% Yield[b]
1	Water	50	12	R.T.	85	10	MeOH	50	12	R.T.	32
2	Water	50	24	R.T.	82	11	DMF	50	12	R.T.	28
3	Water	50	06	R.T.	68	12	DMSO	50	12	R.T.	57
4	Water	50	12	04^0C	48	13	Water	-	12	R.T.	nd[c]
5	Water	50	12	50^0C	52	14	Water	50	12	R.T.	37[d]
6	Water	25	12	R.T.	45	15	(Degassed)Water	50	12	R.T.	Traces[d]
7	Water	100	12	R.T.	81	16	Water	50	12	R.T.	81[e]
8	TBME	50	12	R.T.	22	17	Water	50	12	R.T.	82[f]
9	EtOH	50	12	R.T.	42						

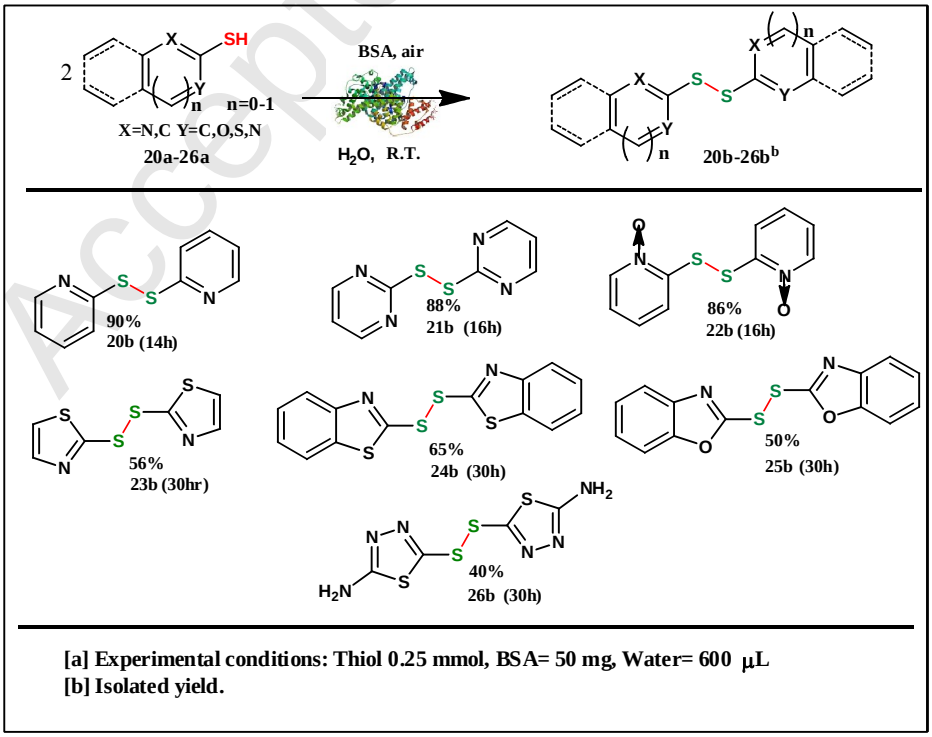
[a] Experimental conditions: 0.25 mmol **1a**, 600 µL solvent; BSA= *Bovine serum albumin* 50 mg; [b] Yield on the basis of GC-MS; [c] without BSA; [d] under N_2 atmosphere; [e] in the presence of BHT (Butylated hydroxytoluene); [f] reaction in dark.

Table 3: Substrate scope for oxidative coupling of aromatic thiols into disulfides in BSA-water-oxygen catalytic system[a]



[a] Reaction conditions: Thiol 0.25 mmol, BSA 50 mg, water 600 µL, rt, reaction time (12-48h); [b[Isolated yield; [c] yield on GC-MS basis.

Table 4: Substrate scope for oxidative coupling of heterocyclic thiols into disulfides in BSA-water-oxygen catalytic system[a]

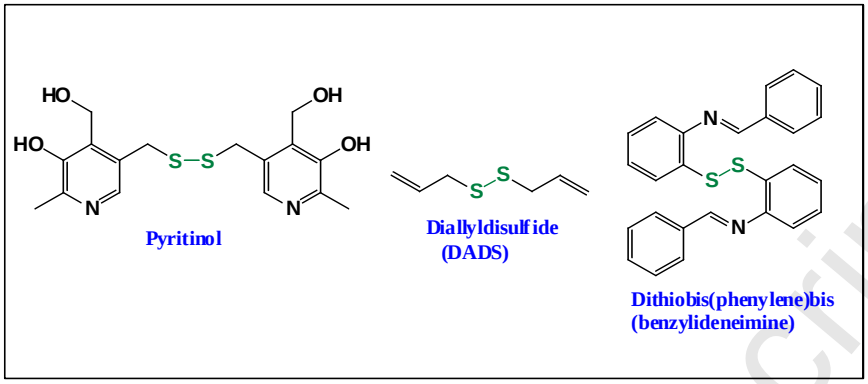


[a] Experimental conditions: Thiol 0.25 mmol, BSA= 50 mg, Water= 600 µL
[b] Isolated yield.

Table 5: Comparison of different protocols for the synthesis of disulphides from thiols with our BSA catalyzed protocol in neutral condition:

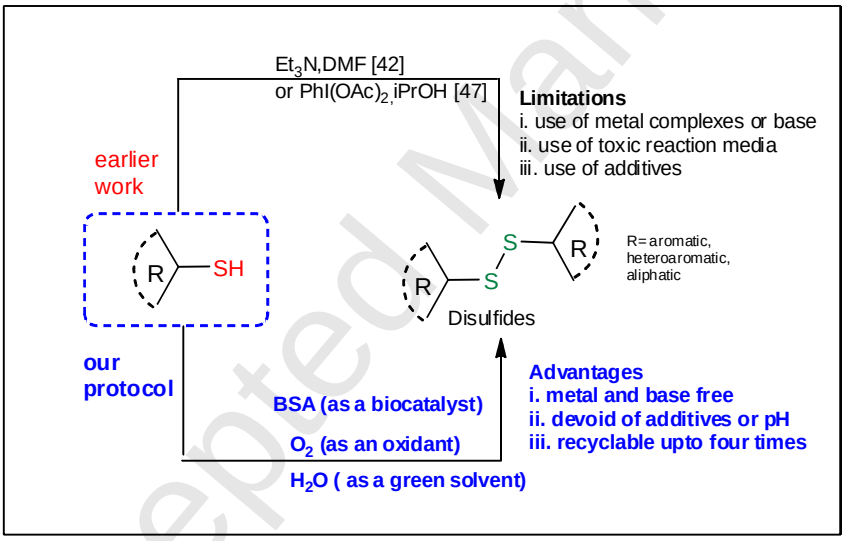
Biocatalyst											
Sr No	**Catalyst**	**Conditions**	**Temp.**	**Solvent**	**Ref**	**Sr No**	**Catalyst**	**Conditions**	**Temp.**	**Solvent**	**Ref**
1	Laccase	pH 4.4	---	MeOH	58	**2**	Horse radish peroxidase	pH 6.0	---	ACN	56
3	Mushroom tyrosinase	pH 6.8	---	ACN	56	**4**	Baker's Yeast	pH 7.2, Incubation at	37^0C	20% EtOH,	57
5	Ethyl Lactate	---	60^0C	Ethyl Lactate	39	**6**	**BSA**	---	**Room temp.**	**"On Water"**	**Our work**
Organocatalyst											
7	Arginine	---	50^0C	Water,	49						
Transition Metal											
8	Fe(BTC)	----	70^0C	MeCN	40	**9**	$Cu(NO_3)_2$. $3H_2O$	---	RT	Acetone	118
10	$[Rh(cod)_2]BF_4$	----	4^0C	DCM	119	**11**	Ni (Nickel) nanoparticles	---	RT	MeCN,	120
12	Cu nanoparticles	----	MW	DMF	121	**13**	Au (Gold) nanoparticles	---	----	----	46
14	Cobalt(II) phthalocyanines	----			122						
Oxidants and other catalysts used											
15	$(NH_4)_2Cr_2O_7$/silica chloride	----	Reflux	DCM	123	**16**	[bmim][$SeO_2(OCH_3)$]	MW	60^0C		124
17	$[Al(NO_3)_3.9H_2O]$ and silica sulfuric acid (SiO_2–OSO_3H)	----	RT	DCM	125, 126	**18**	[hmim][Br]	O_2	50^0C		44
19	HNO_3	----	0^0C	DCM,	127	**20**	2,6-Dicarboxy-pyridinium-chlorochromate	---	RT	MeCN,	131
21	DDQ	----	0^0C	DCM,	128	**22**	K_3PO_4	---	37^0C	MeCN,	132
23	CsF–Celite	----	RT or Reflux	MeCN,	129	**24**	Quantum dots	---	---	---	51
25	Et_3N Sonication	Air Sonication		DMF,	42	**25**	Hypervalent iodine	---	---	---	47
27	Al_2O_3	----	----	----	38	**28**	Diaryl tellurides	Air Sensitizer	0^0C	----	43
29	Ascorbyl radical		RT	Water	130	**30**	Mn(III) Schiff–base		0^0C	MeOH	133

716 **Figures and Schemes**



717

718 **Figure: 1**



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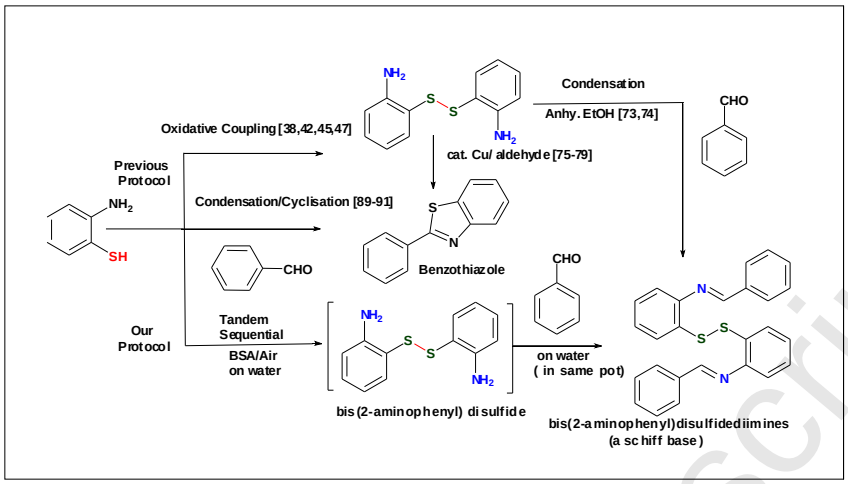
720 **Figure: 2**



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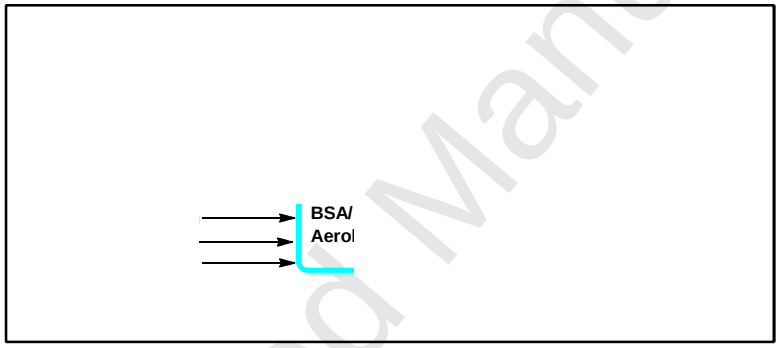
722 **Figure: 3**



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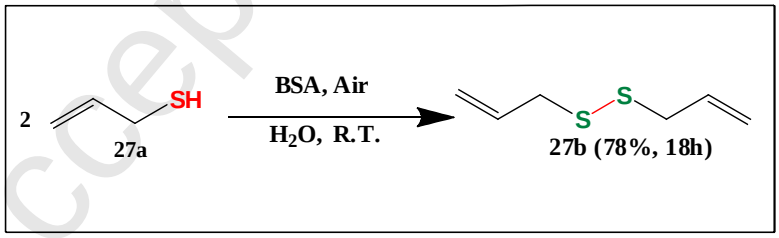
724 **Figure: 4**



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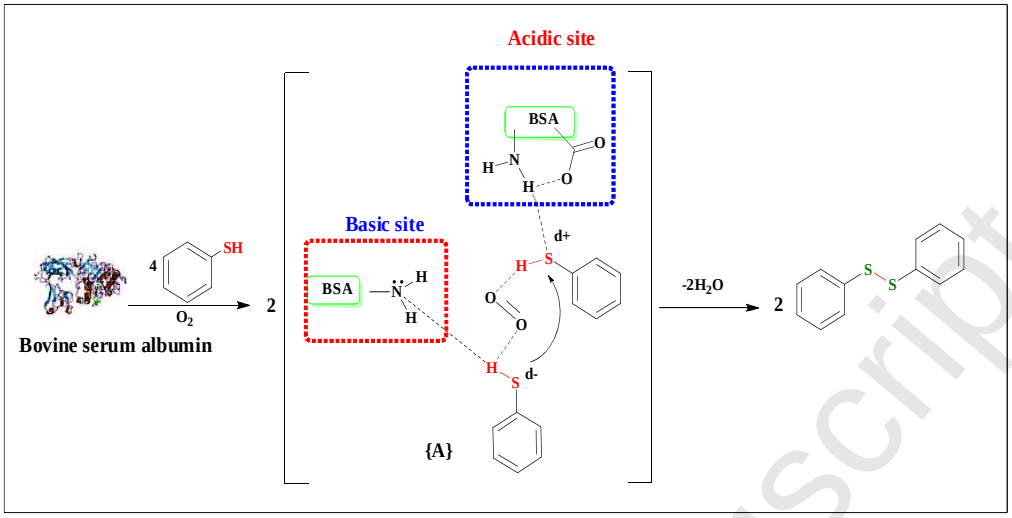
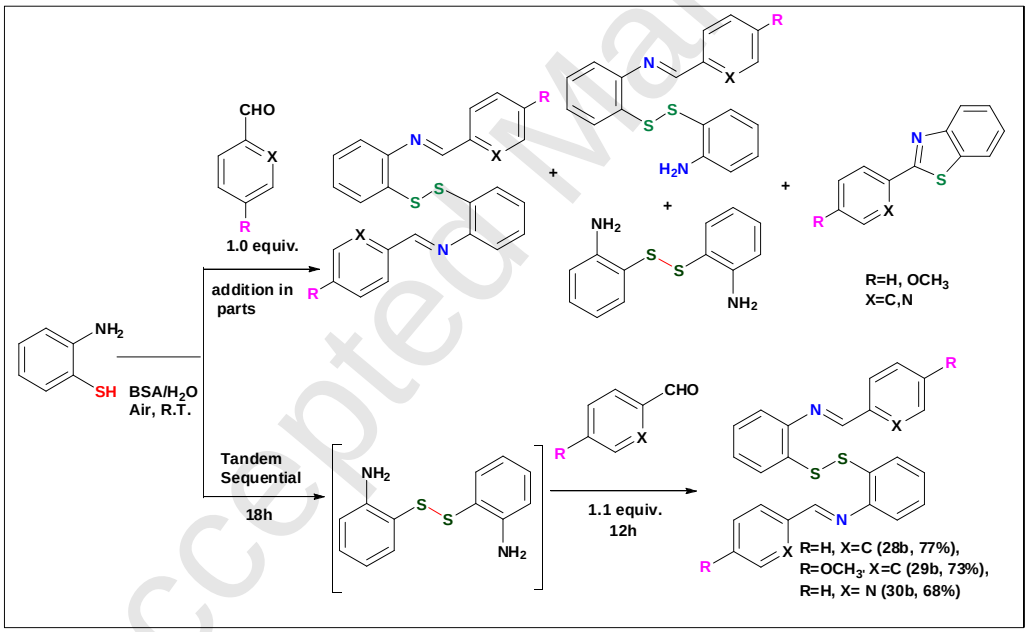
726 **Scheme:1**



Scheme:2



Scheme:3