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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of diamide derivatives of (*S*)-BINOL and biphenyl as potential anti-inflammatory/ anti-arthritic agents

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Abstract A series of (*S*)-BINOL and biphenyl based diamides have been synthesized and characterized from analytic and spectral data. Anti-arthritic activity was studied by inhibition of protein denaturation method (Bovine serum albumin). All the diamides exhibit excellent anti-arthritic activity. Anti-inflammatory activity of the diamides synthesized was investigated using human red blood cells membrane stabilization method, and some of the diamides exhibit good anti-inflammatory activity.

Keywords Diamide · Cysteamine · Anti-inflammatory · Anti-arthritic

Introduction

Difluorobiphenyl amides have been reported as potential anti-inflammatory agents recently (Zhong *et al.*, 2009). The diamides prepared from 2-phenylimidazopyridines are effective in animal models of asthma, cancer, inflammation, and infectives (antiviral) (Banie *et al.*, 2007). Biaryl diamides exhibit as potent melanin concentrating hormone receptor 1 antagonists (Palani *et al.*, 2005). The biphenyl diamides are novel series of p38 MAP kinase inhibitor (Angell *et al.*, 2008a, 2008b). Synthesis of new chiral receptors containing (*S*)-BINOL and thiourea units, and their enantioselective recognition ability for chiral carboxylate anions has been recently reported (Hu *et al.*, 2009).

Inflammation comprises a complex series of biochemical events that can be initiated by a number of stimuli including noxious chemical agents, pathogens, and autoimmune responses, and in pathological situations, may lead to the destruction of cells, tissues, and organs. Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by counteracting the cyclooxygenase (COX) enzyme that converts arachidonic acid into prostaglandins in inflammatory processes (Tozokoparan *et al.*, 2007; Cena *et al.*, 2003). NSAID are used for the treatment of pain, fever, and inflammation particularly arthritis (Bhandari *et al.*, 2008). The common NSAIDs such as aspirin, ibuprofen, naproxen, and fenbufen have side effects like upper GI irritation, ulceration, dyspepsia, bleeding, in some cases deaths and gives only temporary relief for short periods (Zadrazil, 2006; Husain *et al.*, 2009). To overcome the GI ulceration side effect of drug, more COX-2 selective inhibitor NSAID is preferred, which does not significantly inhibit cyclooxygenase in the stomach and appears to be less likely to cause GI ulceration (Laine, 1996). Unfortunately, very effective COX-2 selective inhibitor drugs, i.e., rofecoxib and celecoxib, were withdrawn from the market because of the increased risk of heart attack and stroke associated with long-term, high-dose use. Hence, it is of interest to synthesize and study the anti-arthritic and anti-inflammatory activity of novel amides. However, to the best of our knowledge, such BINOL, biphenyl based diamides have not been reported in the literature. Herein, we report the synthesis, characterization of anti-arthritic and anti-inflammatory activities of amides **1**–**8**.

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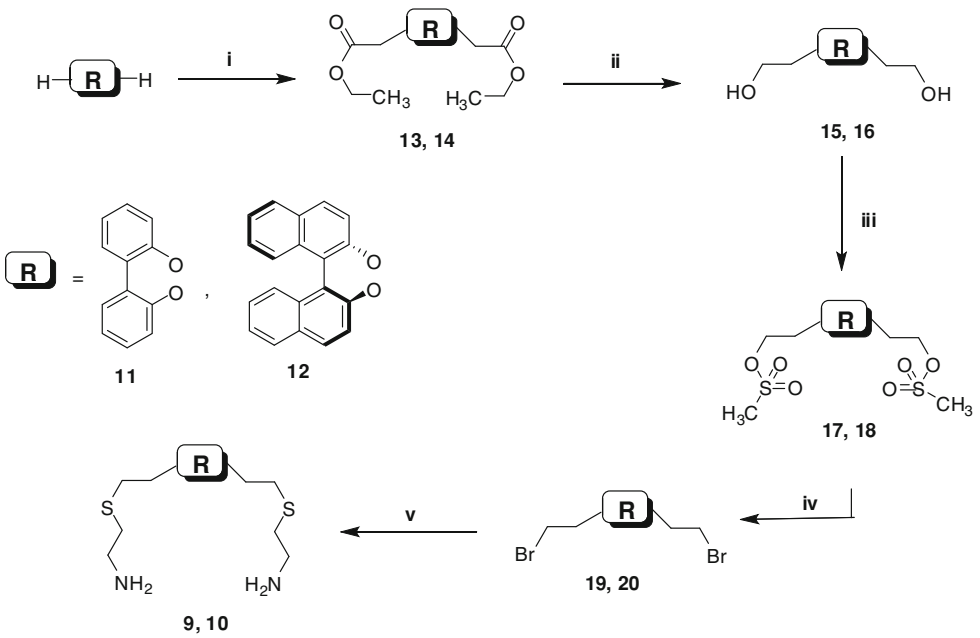
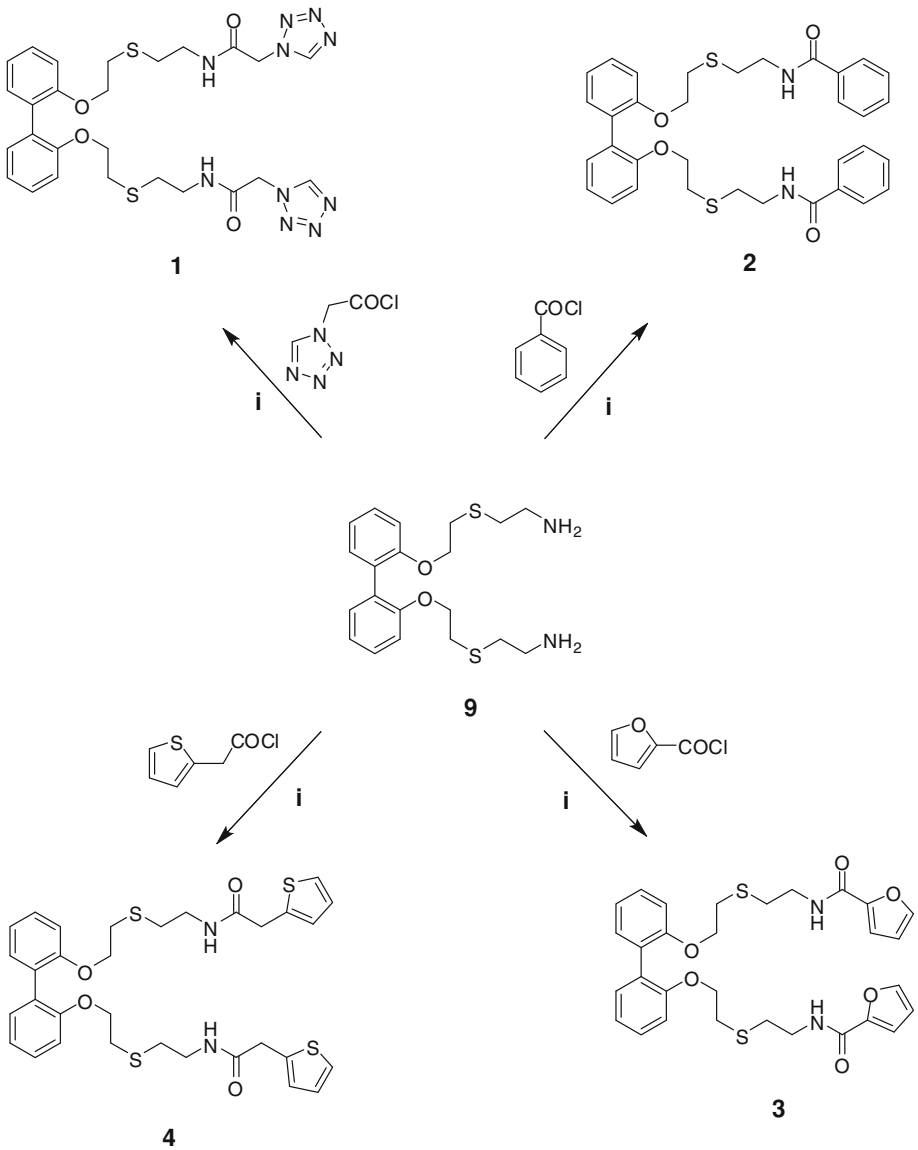
Results and discussion

The synthetic pathway leading to diamines **9** and **10** is outlined in Scheme 1. Reaction of 1.0 equiv. of bisphenol **11** and (*S*)-BINOL **12** with 2.2 equiv. of ethyl bromoacetate in the presence of 3.0 equiv. potassium carbonate in acetonitrile in the presence of catalytic amount of KI at reflux for 8 h resulted in the formation of bis-oxy esters **13** and **14** in about 73 and 70 % yields, respectively, after purification by column chromatography. The structure of the bis-oxy esters **13** and **14** was confirmed from the spectral and analytic data. The ^{1}H NMR spectrum of bis-oxy ester **13** displayed the *O*-methylene protons as a quartet at δ 4.10 and singlet at δ 4.70. The rest of the aromatic protons appeared between δ 6.94 and 7.31. The mass spectrum showed the molecular ion peak ($[M+NH_4]^+$) at *m*/*z* 376.1. Similarly, the structure of bis-oxy ester **14** was confirmed from spectral and analytic data. The bis-oxy esters **13** and **14** were reduced using $LiAlH_4$ (4.0 equiv.) in dry THF at reflux for 3 h. The reaction mixture was quenched with saturated ammonium chloride solution to give the bis-oxy alcohols **15** and **16** with 65 and 70 % yields, respectively. The structure of the bis-oxy alcohols **15** and **16** was confirmed from the spectral and analytic data. The ^{1}H NMR spectrum of bis-oxy alcohol **15** displayed the *O*-methylene protons as a multiplet between δ 3.55–3.60 and triplet at δ 3.94. The rest of the aromatic protons appeared between δ 6.93 and 7.30. The mass spectrum showed the molecular ion peak ($[M+NH_4]^+$) at *m*/*z* 292.1. Similarly, the structure of bis-oxy alcohol **16** was confirmed from spectral and analytic data. The bis-oxy alcohols **15** and **16** were treated with methane sulfonyl chloride 3.0 equiv. in the presence of triethyl amine in dichloromethane at 0–5 °C for 2 h. After usual workup followed by recrystallization from acetone afforded the bis-oxy mesyl compounds **17** and **18** in about 71 and 80 % yields, respectively. The structure of the bis-oxy mesyl compounds **17** and **18** was confirmed from the spectral and analytic data. The ^{1}H NMR spectrum of bis-oxy mesyl compound **17** displayed the *O*-methylene protons as a multiplet between δ 4.21–4.23 and δ 4.37–4.40. The rest of the aromatic protons appeared between δ 6.98 and 7.34. The mass spectrum showed the molecular ion peak ($[M+NH_4]^+$) at *m*/*z* 448.1. Similarly, the structure of bis-oxy mesyl compound **18** was confirmed from spectral and analytic data. Bis-oxy mesyl compounds **17** and **18** were brominated with LiBr 4.0 equiv. in dry THF at room temperature. After usual workup, bis-oxy bromides **19** and **20** were isolated in about 77 and 78 % yields, respectively, after purification by column chromatography. The structure of the bis-oxy bromide **19** and **20** was confirmed from the spectral and analytic data. The ^{1}H NMR spectrum of bis-oxy bromide compound **19** displayed the *O*-methylene protons as a triplet at δ 3.62 and δ 4.25. The rest of the aromatic protons appeared between δ 6.98 and 7.32. The mass spectrum showed the molecular ion peak ($[M+NH_4]^+$) at *m*/*z* 416.0. Similarly, the structure of bis-oxy bromide **20** was confirmed from spectral and analytic data. The bis-oxy bromides **19** and **20** were treated with cysteamine 10 equiv. in dry THF in the presence of sodium methoxide at room temperature for 4 h. The bis-oxy amines **9** and **10** were purified by column chromatography in about 65 and 72 % yields, respectively. The structure of the bis-oxy amines **9** and **10** was confirmed from the spectral and analytic data.



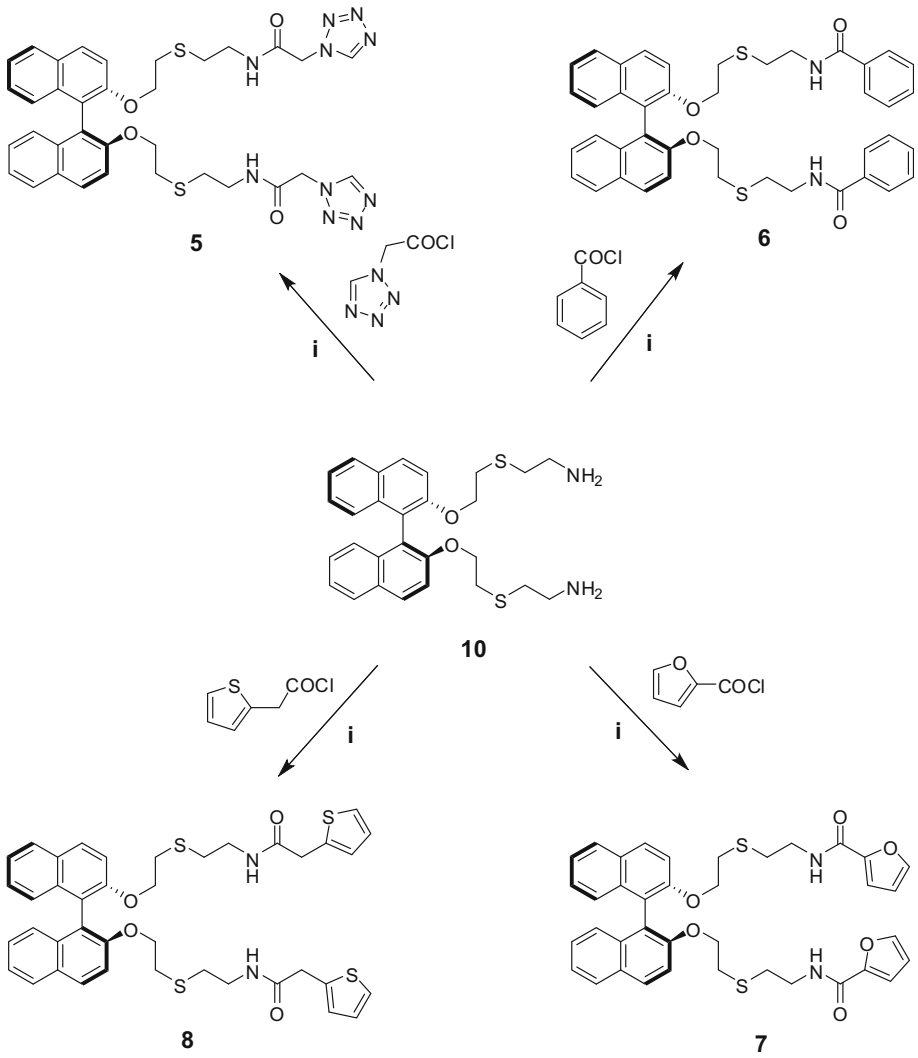
Scheme 1 Reagents and conditions: *i* ethyl bromo acetate, K_2CO_3, KI, ACN, reflux, 8 h, **13** (73 %) and **14** (70 %); *ii* $LiAlH_4$, THF, reflux, 3 h, **15** (65 %) and **16** (70 %); *iii* mesyl chloride, DCM, 0–5 °C, 2 h, **17** (71 %) and **18** (80 %); *iv* LiBr, THF, rt, 4 h, **19** (78 %) and **20** (78 %) and *v* cysteamine, sodium methoxide, THF, rt, 4 h, **9** (65 %) and **10** (72 %)



Scheme 2 Reagents and conditions: *i* TEA, DCM, rt, 3 h, **1** (67 %), **2** (68 %), **3** (71 %) and **4** (71 %)

The ^{1}H NMR spectrum of bis-oxy amine **9** displayed the *N*-CH_2 protons as a triplet at δ 2.68, *O*-methylene protons as a triplet at δ 4.07, and *S*-CH_2 protons as a triplet at δ 2.40 and δ 2.57. The rest of the aromatic protons appeared between δ 6.95 and 7.32. In the ^{13}C NMR spectrum of **9**, the *N*-CH_2 carbons appeared at δ 41.1, *O*-methylene carbons appeared at δ 68.4, and *S*-CH_2 carbons appeared at δ 29.9 and 34.8. The mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m*/*z* 393.1. Similarly, the structure of bis-oxy amine **10** was confirmed from spectral and analytic data. All the new compounds gave satisfactory FT-IR, ^{1}H and ^{13}C NMR, and mass spectral analysis.

In order to test the synthetic utility of bis-oxy amine **9** for the synthesis of diamides, 1.0 equiv. of bis-oxy amine **9** was coupled with 2.2 equiv. of each tetrazole acetyl chloride, benzoyl chloride, furoyl chloride, and thiophene acetyl chloride in the presence of TEA in dry DCM at room temperature. The reaction mixture after usual work-up afforded the diamides **1**–**4** in about 67, 68, 71, and 71 % yields, respectively (Scheme 2). The structure of the bis-oxy diamides **1**–**4** was confirmed from the spectral and analytic data. The ^{1}H NMR spectrum of diamide **1** displayed the *N*-CH_2 protons as a AB quartet at δ 3.12, a singlet at δ 5.22, *O*-methylene protons as a triplet at δ 4.07, *S*-CH_2 protons as a triplet at δ 2.43, 2.70, and NH protons as a triplet at δ 8.46. The rest of the aromatic protons appeared between δ 6.95 and 9.33. In the ^{13}C NMR spectrum of **8**, the *N*-CH_2 carbons appeared at δ 38.6, 49.4; *O*-methylene carbons appeared at δ 68.4; *S*-CH_2 carbons appeared at δ 29.9, 31.0; and carbonyl carbon at δ 164. The FT-IR spectrum of **1** showed the carbonyl carbon stretching frequency at 1,671 cm^{-1}, and the mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m*/*z* 613.1. Similarly, the structures of diamides **2**–**4** were confirmed from



Scheme 3 Reagents and conditions: *i* TEA, DCM, rt, 3 h, **5** (71 %), **6** (66 %), **7** (67 %) and **8** (62 %)

Table 1 In vitro anti-arthritic activity of diamides **1**–**8** by inhibition of protein denaturation method (Bovine serum albumin)

Diamide	Activity (% inhibition of protein denaturation)				
	50 μg/mL	100 μg/mL	200 μg/mL	400 μg/mL	800 μg/mL
1	21.6	42.2	65.1	76.3	91.9
2	15.8	38.2	53.0	67.8	81.8
3	25.7	46.6	68.8	83.1	97.2
4	28.3	34.9	41.1	69.6	84.8
5	19.6	39.6	67.1	74.1	94.2
6	19.5	30.3	52.3	65.8	82.7
7	29.5	50.8	71.5	87.8	98.7
8	23.6	42.2	59.1	73.3	87.4
Diclofenac sodium	5.6	10.9	15.2	21.6	76.7

Each value represents mean ± SD of three observations

spectral and analytic data. All the new compounds gave satisfactory FT-IR, ^{1}H and ^{13}C NMR, mass spectral, and elemental analysis.

In order to test the synthetic utility of bis-oxy amine **10** for the synthesis of diamides, 1.0 equiv. of bis-oxy amine **10** was coupled with 2.2 equiv. of each tetrazole acetyl chloride, benzoyl chloride, furoyl chloride, and thiophene acetyl chloride in the presence of TEA in dry DCM at room temperature. The reaction mixture, after usual work-up, afforded the diamides **5**–**8** in about 71, 66, 67, and 62 % yields, respectively (Scheme 3). The structure of the bis-oxy diamides **5**–**8** was confirmed from the spectral and analytic data. The ^{1}H NMR spectrum of diamide **5** displayed the *N*-CH_2 protons as a multiplet between δ 2.90 and 2.97, a singlet at δ 5.20, *O*-methylene protons as a triplet at δ 4.14, *S*-CH_2 protons as a multiplet between δ 2.13–2.23, at δ 2.50 (merged with DMSO), and NH protons

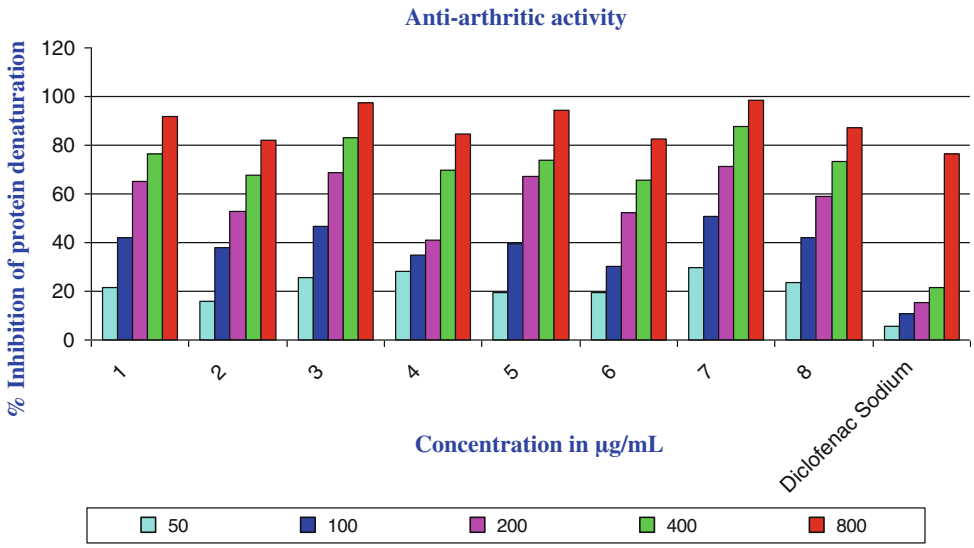


Fig. 1 Anti-arthritic activity of diamides **1–8**

as a triplet at δ 8.38. The rest of the aromatic protons appeared between δ 6.92 and 9.34. In the ^{13}C NMR spectrum of **5**, the *N*-CH_2 carbons appeared at δ 38.2, 49.3; *O*-methylene carbons appeared at δ 69.7; *S*-CH_2 carbons appeared at δ 29.9, 30.7; and carbonyl carbon at δ 164.5. The FT-IR spectrum of **5** showed the carbonyl carbon stretching frequency at 1,676 cm^{-1}, and the mass spectrum showed the molecular ion peak ($[M+H]^+$) at *m/z* 713.2. Similarly, the structures of diamides **6–8** were confirmed from spectral and analytic data. All the new compounds gave satisfactory FT-IR, ^{1}H and ^{13}C NMR, mass spectral, and elemental analysis.

Anti-arthritic activity

In order to study the anti-arthritic activity of the diamides **1–8**, inhibition of protein denaturation method [Bovine serum albumin (BSA)] (Sangeetha *et al.*, 2011; Mizushima and Kobayashi, 1968; Mizushima, 1965) was employed, and diclofenac sodium was used as a standard. The denaturation of protein as one of the causes for rheumatoid arthritis was well documented (Mizushima, 1966). Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins (Brown and Mackey, 1968). The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic, and disulfide bonding (Grant *et al.*, 1970). The anti-arthritic activities of all the diamides are concentration-dependent. At higher concentration, the amides exhibit better anti-arthritic activity than at lower concentration. The amides **7**, **3**, **5**, **1**, **8**, **4**, **6** and **2** were found to possess the maximum anti-arthritic activity (98.7, 97.2, 94.2, 91.9, 87.4, 84.8, 82.7, and 81.8 % at 800 µg/mL) when compared to the reference drug diclofenac sodium (76.7 % at 800 µg/mL), which clearly shows that amides are superior to the reference drug diclofenac sodium. The superior inhibition of protein denaturation results showed that the amides are more stable in BSA than diclofenac sodium. It is possible that the stability related to the strong binding of the amides on BSA is due to the increased hydrophobicity as compared to diclofenac sodium. The degree of anti-arthritic activity of amides **7**, **3**, **5**, **1**, **8**, **4**, **6** and **2** at 800 µg/mL is found to be 98.7, 97.2, 94.2, 91.9, 87.4, 84.8, 82.7, and 81.8 %, respectively (Table 1; Fig. 1).

Anti-inflammatory activity

Further anti-inflammatory activity of compounds **1–8** was investigated using human red blood cells (HRBCs) membrane stabilization (Sangeetha *et al.*, 2011; Gandhidsan *et al.*, 1991; Sadique *et al.*, 1989) with prednisolone as the standard, and the results are shown in Table 2. The lysosomal enzymes released during inflammatory condition produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic

Table 2 In vitro anti-inflammatory activity of diamides **1–8** by HRBC membrane stabilization method

Diamide	Activity (% prevention of lysis)			
	10 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
1	54.0 ± 0.2	63.3 ± 0.4	75.3 ± 0.5	89.4 ± 0.3
2	35.7 ± 0.7	49.0 ± 0.4	60.7 ± 0.7	77.5 ± 0.5
3	55.2 ± 0.6	62.9 ± 0.3	84.7 ± 0.5	94.1 ± 0.8
4	47.3 ± 0.8	58.6 ± 0.5	67.2 ± 0.7	83.3 ± 0.6
5	61.9 ± 0.9	69.7 ± 0.5	82.5 ± 0.6	91.6 ± 0.3
6	34.5 ± 0.5	51.4 ± 0.4	63.6 ± 0.6	79.4 ± 0.8
7	56.5 ± 0.4	65.5 ± 0.3	88.9 ± 0.1	96.2 ± 0.4
8	50.2 ± 0.6	61.7 ± 0.4	72.8 ± 0.6	86.0 ± 0.8
Prednisolone	46.0 ± 0.1	57.9 ± 0.3	84.8 ± 0.4	91.0 ± 0.5

Each value represents mean ± SD of three observations

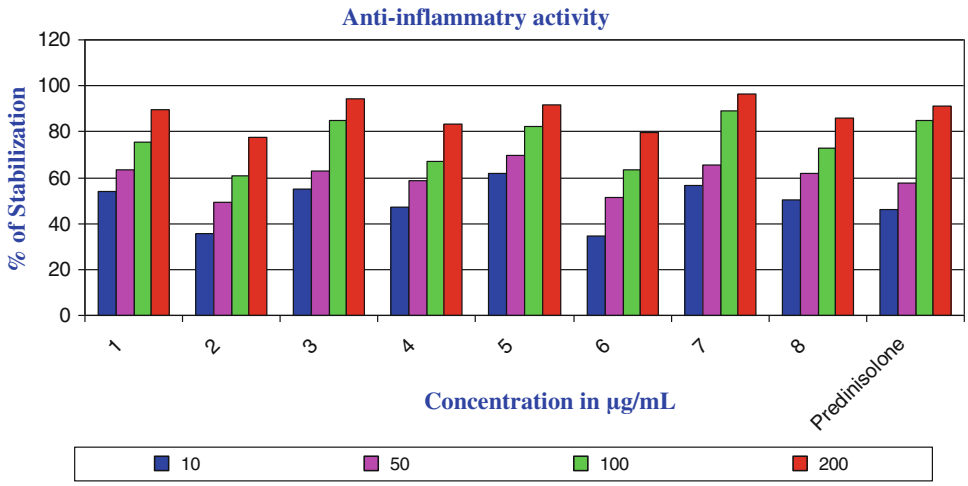


Fig. 2 Anti-inflammatory activity of diamides **1–8**

inflammation. The anti-inflammatory agent acts by either inhibiting the lysosomal enzymes or by stabilizing the lysosomal membranes.

The anti-inflammatory activities of all the amides are concentration-dependent. At higher concentration, the amides exhibited better anti-inflammatory activity than at lower concentration. The amides **7**, **3** and **5** were found to possess the maximum anti-inflammatory activity (96.2, 94.1, and 91.6 % at 200 µg/mL) when compared to the reference drug prednisolone (91.0 % at 200 µg/ml), which clearly shows that anti-inflammatory activity of the amides is superior to the reference drug prednisolone. The better anti-inflammatory activity of amides could be due to their binding on to the erythrocyte membranes with subsequent alteration of the charges on the membrane surface of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the hemolysis of red blood cells. The degree of anti-inflammatory activity of cyclophane amides **7**, **3**, **5**, **1**, **8**, **4**, **6** and **2** at 200 µg/mL is found to be 96.2, 94.1, 91.6, 89.4, 86.0, 83.3, 79.4, and 77.5 %, respectively (Table 2; Fig. 2).

Conclusions

In conclusion, diamides were **1–8** synthesized and show better anti-arthritic activity than the reference drug diclofenac sodium. All the diamides **3**, **5** and **7** show good anti-inflammatory activity at (200 µg/mL) than the reference drug prednisolone. The diamides **1–8** may be developed as anti-arthritic drug as they showed better activity than the reference drug diclofenac sodium. The diamides **3**, **5** and **7** may be developed as anti-inflammatory drug (NSAID) as they show better activity than the reference drug prednisolone. Further studies are required to determine their toxicity, bioavailability, mode of action etc. Synthesis of similar diamides with different biologically important substituent to improve the solubility and efficacy, in vivo anti-arthritic, anti-inflammatory assay, and molecular recognition toward various biologically important anions are under investigation.

Experimental

All the reagents and solvents employed were of the best grade available and were used without further purification. The melting points were determined using a Metler Toledo melting point apparatus by open capillary tube method and were uncorrected. Spectroscopic data were recorded by the following instruments: UV/Vis: Shimadzu 2550 spectrophotometer. IR: Perkin-Elmer series 2000 FT-IR spectrophotometer. NMR: Bruker Avance 400 MHz. Mass: ESI—PerkinElmer Sciex, API 3000 mass spectrometer and FAB—mass spectra Jeol SX 102/DA-6000 mass spectrometer. The elemental analysis for the compounds was carried out using the Elementar Vario EL III elemental analyzer (SIPRA LABS LTD., Hyderabad, India). Precoated silica gel plates from Merck were used for TLC. Column chromatography was carried out using silica gel (100–200 mesh) purchased from ACME.

General procedure for the synthesis of diamines

To a solution of cysteamine (200 mmol), sodium methoxide (210 mmol) in dry THF (100 mL) and dibromide (20 mmol) were added to the reaction mixture and stirred for further 4 h. The solvent was removed at reduced pressure; and the residue obtained was then dissolved in DCM (50 mL), washed with water (2 × 200 mL) and then dried over anhydrous potassium carbonate. Removal of the DCM under reduced pressure gave the corresponding

amine as a crude material which was purified by column chromatography (SiO_2).

*2,2′-(2,2′-(Biphenyl-2,2′-diylbis(oxy))bis(ethane-2, 1-diyl))bis(sulfanediyldiethanamine (**9**)*

Yield 65 %; mp 93 °C; IR (KBr, cm^{-1}) 1669, 3360; ^1H NMR (400 MHz, DMSO-d_6)δ 7.27–7.32 (m, 2H), 7.16 (dd, 2H, $J = 7.4$ and 1.6 Hz), 7.04 (d, 2H, $J = 8.1$ Hz), 6.95 (t, 2H $J = 7.4$ Hz), 4.07 (t, 4H, $J = 6.5$ Hz), 2.68 (t, 4H, $J = 6.5$ Hz), 2.57 (t, 4H, $J = 6.6$ Hz), 2.40 (t, 4H, $J = 6.6$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.7, 131.2, 128.6, 127.6, 120.3, 112.2, 68.4, 41.1, 34.8, 29.9; MS (ES) *m/z*: 393.1 $[M+H]^+$; Elemental Anal. Calc. for. $C_{20}H_{28}N_2O_2S_2$: C, 61.19; H, 7.19; N, 7.14; S, 16.34 %; Found: C, 61.43; H, 7.35; N, 7.31; S, 16.18 %.

*(S)-2,2′-(2,2′-(1,1′-Binaphthyl-2,2′-diylbis(oxy))bis (ethane-2,1-diyl))bis(sulfanediyl) diethanamine (**10**)*

Yield 72 %; mp 96 °C; IR (KBr, cm^{-1}) 1634, 3350; ^1H NMR (400 MHz, DMSO-d_6) δ 8.06 (m, 2H), 7.95 (d, 2H, $J = 8.1$ Hz), 7.62 (d, 2H, $J = 9.1$ Hz), 7.33–7.37 (m, 2H), 7.23–7.27 (m, 2H), 6.93 (d, 2H $J = 8.4$ Hz), 4.14–4.24 (m, 4H), 2.62–2.71 (m, 4H), 2.53–2.61 (m, 4H), 2.40–2.50 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.7, 133.4, 129.5, 128.9, 128.0, 126.4, 124.6, 123.6, 119.3, 115.7, 69.2, 37.9, 29.7, 28.2; MS (ES) *m/z*: 493.3 $[M+H]^+$; Elemental Anal. Calc. for. $C_{28}H_{32}N_2O_2S_2$: C, 68.26; H, 6.55; N, 5.69; S, 13.02 %; Found: C, 68.11; H, 6.45; N, 5.81; S, 13.18 %.

General procedure for the synthesis of diamides

To a solution of diamine (1.0 mmol), TEA (2.5 mmol) in dry DCM (20 mL) and acid chloride (2.2 mmol) were added to the reaction mixture and stirred for 3 h. The reaction mixture was washed with water to remove triethylammonium chloride and then dried over anhydrous sodium sulfate. Removal of the DCM under reduced pressure gave the corresponding amide as a crude material which was purified by column chromatography (SiO_2).

*N-(2-(2-(2′-(2-(2-(2-Oxo-2-(1H-tetrazol-1-yl) ethylamino)ethylthio)ethoxy)biphenyl-2-yloxy) ethylthio) ethyl)-2-(1H-tetrazol-1-yl)acetamide (**1**)*

Yield 67 %; mp 167 °C; IR (KBr, cm^{-1}) 1671, 3304; ^1H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 2H), 8.46 (t, 2H, $J = 5.1$ Hz), 7.27 (t, 2H, $J = 7.9$ Hz), 7.16 (t, 4H, $J = 7.4$ Hz), 7.02 (d, 2H, $J = 8.3$ Hz), 6.95 (t, 2H, $J = 7.3$ Hz), 5.22 (s, 4H), 4.07 (t, 4H, $J = 6.2$ Hz), 3.12 (q, 4H, $J = 6.2$ Hz), 2.70 (t, 4H, $J = 6.2$ Hz), 2.43 (t, 4H, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 155.7, 145.1, 131.2, 128.6, 127.5, 120.4, 112.20, 68.4, 49.4, 38.6, 31.0, 29.9; MS (ES) *m/z*: 613.1 $[M+H]^+$; Elemental Anal. Calc. for. $C_{26}H_{32}N_{10}O_4S_2$: C, 50.97; H, 5.26; N, 22.86; S, 10.47 %; Found: C, 50.78; H, 5.35; N, 22.72; S, 10.38 %.

*N,N′-(2,2′-(2,2′-(Biphenyl-2,2′-diylbis(oxy))bis (ethane-2,1-diyl))bis(sulfanediyl)bis(ethane-2,1-diyl)) dibenzamide (**2**)*

Yield 68 %; mp 118 °C; IR (KBr, cm^{-1}) 1633, 3317; ^1H NMR (400 MHz, DMSO-d_6) δ 8.50 (bs, 2H), 7.81 (d, 4H, $J = 7.4$ Hz), 7.50 (d, 2H, $J = 7.0$ Hz), 7.43 (t, 4H, $J = 7.6$ Hz), 7.22 (t, 2H, $J = 7.9$ Hz), 7.16 (d, 2H, $J = 7.4$ Hz), 7.01 (d, 2H, $J = 8.2$ Hz), 6.92 (t, 2H $J = 7.4$ Hz), 4.10 (t, 4H, $J = 6.3$ Hz), 3.29 (d, 4H, $J = 7.4$ Hz), 2.74 (t, 4H, $J = 6.2$ Hz), 2.55 (t, 4H, $J = 6.7$ Hz),; ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 155.7, 134.4, 131.2, 131.1, 128.5, 128.3, 127.5, 127.1, 120.3, 112.2, 68.3, 40.1*, 31.1, 29.9, *Merged with DMSO peak; MS (ES) *m/z*: 600.9 $[M+H]^+$; Elemental Anal. Calc. for. $C_{34}H_{36}N_2O_4S_2$: C, 67.97; H, 6.04; N, 4.66; S, 10.67 %; Found: C, 67.78; H, 6.22; N, 4.72; S, 10.48 %.

*N,N′-(2,2′-(2,2′-(Biphenyl-2,2′-diylbis(oxy))bis (ethane-2,1-diyl))bis(sulfanediyl)bis(ethane-2,1-diyl)) bis(2-(thiophen-2-yl)acetamide)(**3**)*

Yield 71 %; mp 100 °C; IR (KBr, cm^{-1}) 1649, 3277; ^1H NMR (400 MHz, DMSO-d_6)δ 8.09 (t, 2H, $J = 5.5$ Hz), 7.32 (dd, 2H, $J = 5.12$ and 1.2 Hz), 7.25–7.29 (m, 2H), 7.15 (dd, 2H, $J = 7.5$ and 1.7 Hz), 7.01 (d, 2H, $J = 8.0$ Hz), 6.92–6.97 (m, 4H), 6.89–6.90 (m, 2H), 4.06 (t, 4H, $J = 6.4$ Hz), 3.62 (s, 4H), 3.08 (q, 4H, $J = 6.6$ Hz), 2.68 (t, 4H, $J = 6.4$ Hz), 2.42 (t, 4H, $J = 6.9$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.0, 155.7, 137.6, 131.2, 128.5, 127.5, 126.5, 126.0, 124.8, 120.3, 112.2, 68.3, 38.6*, 36.5, 31.1, 30.0, *Merged with DMSO peak; MS (ES) *m/z*: 640.8 $[M+H]^+$; Elemental Anal. Calc. for. $C_{32}H_{36}N_2O_4S_4$: C, 59.97; H, 5.66; N, 4.37; S, 20.01 %; Found: C, 59.82; H, 5.53; N, 4.60; S, 20.23 %.

*N,N′-(2,2′-(2,2′-(Biphenyl-2,2′-diylbis(oxy)) bis(ethane-2,1-diyl))bis(sulfanediyl)bis(ethane-2,1-diyl)) difuran-2-carboxamide (**4**)*

Yield 71 %; mp 115 °C; IR (KBr, cm^{-1}) 1642, 3287; ^1H NMR (400 MHz, DMSO-d_6)δ 8.31 (t, 2H, $J = 5.6$ Hz), 7.82 (s, 2H), 7.21 (t, 2H, $J = 7.4$ Hz), 7.15 (d, 2H, $J = 7.3$ Hz), 7.07–7.08 (m, 2H), 7.01 (d, 2H, $J = 8.2$ Hz), 6.92 (d, 2H, $J = 7.4$ Hz), 6.61–6.62 (m, 2H), 4.09 (t, 4H, $J = 6.4$ Hz), 3.23 (t, 4H, $J = 6.5$ Hz), 2.72 (t, 4H, $J = 6.4$ Hz), 2.53–2.54

(m, 4H)*, *merged with DMSO peak; ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.6, 155.7, 147.9, 144.9, 131.2, 128.5, 127.5, 120.3, 113.3, 112.2, 111.9, 68.3, 38.1, 31.1, 29.9; MS (ES) *m/z*: 580.9 $[M+H]^+$; Elemental Anal. Calc. for. $C_{30}H_{32}N_2O_6S_2$: C, 62.05; H, 5.55; N, 4.82; S, 11.04 %; Found: C, 62.32; H, 5.47; N, 4.66; S, 11.23 %.

*(S)-N,N′-(2,2′-(2,2′-(1,1′-Binaphthyl-2,2′-diylbis(oxy)) bis(ethane-2,1-diyl))bis (sulfanediyl)bis(ethane-2,1-diyl)) bis(2-(1H-tetrazol-1-yl)acetamide (**5**)*

Yield 75 %; mp 170 °C; IR (KBr, cm^{-1}) 1676, 3289; ^{1}H NMR (400 MHz, DMSO-d_6)δ 9.34 (s, 2H), 8.38 (t, 2H, J = 5.24 Hz), 8.03 (d, 2H, J = 9.04 Hz), 7.93 (d, 2H, J = 8.20 Hz), 7.57 (d, 2H, J = 9.08 Hz), 7.31 (t, 2H, J = 7.16 Hz), 7.21 (t, 2H, J = 7.88 Hz), 6.92 (d, 2H, J = 8.44 Hz), 5.20 (s, 4H), 4.14 (t, 4H, J = 6.24 Hz), 2.90–2.97 (m, 4H), 2.13–2.23 (m, 4H), 4-CH_2 Protons merged with DMSO peak; ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 153.8, 145.1, 133.4, 129.4, 128.9, 127.9, 126.3, 124.6, 123.6, 119.2, 115.6, 69.7, 49.3, 38.2, 30.7, 29.9; MS (ES) *m/z*: 713.2 $[M+H]^+$; Elemental Anal. Calc. for. $C_{34}H_{36}N_{10}O_4S_2$: C, 57.29; H, 5.09; N, 19.65; S, 9.00 %; Found: C, 57.41; H, 5.23; N, 19.76; S, 9.13 %.

*(S)-N,N′-(2,2′-(2,2′-(1,1′-Binaphthyl-2,2′-diylbis(oxy)) bis(ethane-2,1-diyl))bis (sulfanediyl)bis(ethane-2,1-diyl)) dibenzamide (**6**)*

Yield 66 %; mp 107 °C; IR (KBr, cm^{-1}) 1651, 3318; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41–8.42 (m, 2H), 8.00 (d, 2H, J = 8.9 Hz), 7.90 (d, 2H, J = 7.8 Hz), 7.80 (d, 4H, J = 7.2 Hz), 7.57 (d, 2H, J = 9.0 Hz), 7.43–7.51 (m, 6H), 7.14–7.33 (m, 4H), 6.93 (d, 2H, J = 8.3 Hz), 4.17–4.20 (m, 4H), 3.12–3.15 (m, 4H), 2.53–2.56 (m, 4H)*, 2.29–2.39 (m, 4H), *merged with DMSO peak; ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 134.8, 133.8, 131.5, 129.8, 129.3, 128.6, 128.4, 127.5, 126.7, 125.0, 123.9, 119.7, 116.0, 69.9, 39.0, 31.2, 30.3; MS (ES) *m/z*: 700.9 $[M+H]^+$; Elemental Anal. Calc. for. $C_{32}H_{40}N_2O_4S_2$: C, 71.97; H, 5.75; N, 4.00; S, 9.15 %; Found: C, 71.85; H, 5.57; N, 4.12; S, 9.22 %.

*(S)-N,N′-(2,2′-(2,2′-(1,1′-Binaphthyl-2,2′-diylbis(oxy)) bis(ethane-2,1-diyl))bis (sulfanediyl)bis(ethane-2,1-diyl)) bis(2-(thiophen-2-yl)acetamide) (**7**)*

Yield 62 %; mp 115 °C; IR (KBr, cm^{-1}) 1646, 3408; ^{1}H NMR (400 MHz, DMSO-d_6)δ 8.00–8.04 (m, 4H), 7.92 (d, 2H, J = 8.2 Hz), 7.55 (d, 2H, J = 9.0 Hz), 7.30–7.34 (m, 4H), 7.20 (t, 2H, J = 7.9 Hz), 6.91–6.93 (m, 4H), 6.87–6.88 (m, 2H), 4.12 (t, 4H, J = 6.5 Hz), 3.58 (s, 4H), 2.89–2.92 (m, 4H), 2.15–2.22 (m, 4H), 4-CH_2 protons merged with DMSO peak; ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.9, 153.8, 137.6, 133.4, 129.4, 128.9, 128.0, 126.5, 126.3, 126.0, 124.8, 124.6, 123.6, 119.3, 115.7, 69.5, 38.3, 36.4, 30.9, 29.9; MS (ES) *m/z*: 740.8 $[M+H]^+$; Elemental Anal. Calc. for. $C_{40}H_{40}N_2O_4S_4$: C, 64.83; H, 5.44; N, 3.78; S, 17.31 %; Found: C, 64.69; H, 5.56; N, 3.61; S, 17.22 %.

*(S)-N,N′-(2,2′-(2,2′-(1,1′-Binaphthyl-2,2′-diylbis(oxy)) bis(ethane-2,1-diyl))bis (sulfanediyl)bis(ethane-2,1-diyl)) difuran-2-carboxamide (**8**)*

Yield 67 %; mp 90 °C; IR (KBr, cm^{-1}) 1650, 3290; ^{1}H NMR (400 MHz, DMSO-d_6)δ 8.21 (t, 2H, J = 5.6 Hz), 8.00 (d, 2H, J = 9.0 Hz), 7.90 (d, 2H, J = 8.1 Hz), 7.81–7.82 (m, 2H), 7.56 (d, 2H, J = 9.1 Hz), 7.30 (d, 2H, J = 7.1 Hz), 7.20 (t, 2H, J = 7.6 Hz), 7.05–7.06 (m, 2H), 6.92 (d, 2H, J = 8.4 Hz), 6.60–6.62 (m, 2H), 4.17 (t, 4H, J = 6.5 Hz), 3.03–3.13 (m, 4H), 2.53 (m, 4H)*, 2.22–2.36 (m, 4H), *merged with DMSO peak; ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.6, 153.7, 147.9, 144.9, 133.4, 129.4, 128.9, 128.0, 126.3, 124.6, 123.5, 119.2, 115.6, 113.3, 118.8, 69.5, 37.8, 30.80, 29.8; MS (ES) *m/z*: 740.8 $[M+H]^+$; Elemental Anal. Calc. for. $C_{38}H_{36}N_2O_6S_4$: C, 67.04; H, 5.33; N, 4.11; S, 9.42 %; Found: C, 67.19; H, 5.46; N, 4.26; S, 9.26 %.

Biology

In vitro anti-arthritic study

The test solution (0.5 mL) consists of Bovine Serum Albumin (0.45 mL, 5 % w/v aqueous solution) and a solution of amides in DMSO (800, 400, 200, 100, 50 μg in 0.05 mL). Test control solution (0.5 mL) consists of Bovine Serum Albumin (0.45 mL, 5 % w/v aqueous solution) and distilled water (0.05 mL). Product control solution (0.5 mL) consists of distilled water (0.45 mL) and a solution of amides in DMSO (800, 400, 200, 100, 50 μg in 0.05 mL). Standard solution (0.5 mL) consists of bovine serum albumin (0.45 mL, 5 % w/v aqueous solution) and diclofenac sodium (800, 400, 200 μg/mL in 0.05 mL). All the above solutions were adjusted to pH 6.3 using 1 N HCl. The samples were incubated at 37 °C for 20 min, and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, phosphate buffer (2.5 mL) was added to the above solutions. The absorbance was measured using UV–Visible spectrophotometer at 416 nm. The percentage inhibition of protein denaturation can be calculated as follows:

$$\%\text{Inhibition} = 100 - [\text{optical density of test solution} - \textit{opticaldensityof}\,\text{product control}) \div \text{optical density of test control})] \times 100$$

In vitro anti-inflammatory study

Blood samples were collected from healthy volunteers, and the collected blood was mixed with equal volume of sterilized Alsever's solution (2 % dextrose, 0.8 % sodium citrate, 0.05 % citric acid, and 0.42 % sodium chloride). The blood was centrifuged at 1,500 rpm, and the packed cells were washed with isotonic sodium chloride (0.85 %, pH 7.2) and a 10 % v/v suspension of the packed cells was made with isotonic sodium chloride. The assay mixture contained the amide concentration in DMSO (10, 50, 100, 200, and 400 μg/mL), Phosphate buffer (1 mL, 0.15 M, pH 7.4), hypotonic sodium chloride (2 mL, 0.36 %), and HRBC suspension (0.5 mL). Prednisolone (100 μg) was used as the reference drug. Instead of hypotonic sodium chloride, distilled water (2 mL) was used in the control. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer (Systronic UV–Vis Spectrophotometer 118) at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated using the following formula:

$$\%\text{Protection} = 100 - \{[(\text{optical density of test solution} - \text{optical density ofproduct control}) \div (\text{optical density of test control})]\} \times 100$$

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