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Research Article

Synthesis of some new 1,2,4-triazole derivatives, investigation of their fluorescence properties and biological activities

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1,2,4-Triazoles,
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carbonic anhydrase inhibitory activity.

ABSTRACT

In this work, new heterocyclic compounds containing 1,2,4-triazole ring were synthesized starting from iminoester hydrochlorides and benzhydrazide. The fluorescence properties of the novel triazole compounds synthesized were determined in organic solvents such as methanol and DMSO. The diimine derivatives among the new triazoles show high fluorescence intensities in methanol. Reduced diimines (**8**) have lower fluorescence properties in methanol than diimine compounds (**6**). The compounds were also tested for their antioxidant and carbonic anhydrase inhibitory (CAI) activities. Low CAI activity was observed. Bis Schiff bases (**6**) showed better activity with lower IC_{50} values of 200 and 330 µM. The compounds showed moderate to high antioxidant activity in both DPPH and FRAP antioxidant tests. The SC_{50} values in DPPH test were as low as 0.01 mg/mL, close to that of standard antioxidants. Similarly, a high TEAC value as 8197 mM was observed showing considerably high FRAP activity. Structural differences were effective on the antioxidant activities of the new compounds. Benzoylhydrazones (**2**), oxadiazoles (**3**) and amino-triazoles (**4**) showed higher activity in both antioxidant tests as compared to compounds **5**-**8**.

TR

Bazı yeni 1,2,4-triazol türevlerinin sentezi, floresans özellikleri ve biyolojik aktivitelerinin incelenmesi

Anahtar Kelimeler:
1,2,4-triazol,
Schiff bazı,
antioksidan,
floresans,
karbonik anhidraz inhibitör aktivitesi.

ÖZET

Bu çalışmada, iminoester hidroklorürler ve benzhidrazidden başlanarak 1,2,4-triazol halkası içeren yeni heterosiklik bileşikler sentezlendi. Triazol bileşiklerinin floresans özellikleri metanol ve DMSO gibi organik çözücülerde belirlendi. Triazol bileşikleri arasında diimin bileşikleri metanolde yüksek floresans şiddeti gösterdi. İndirgenmiş bileşikler (**8**) dimin bileşiklerine (**6**) göre metanolde düşük floresans özelliğine sahiptir. Yeni sentezlenen bileşiklerin antioksidan ve karbonik anhidraz inhibitör (CAI) aktiviteleri incelendi. Bileşiklerde düşük CAI aktivitesi gözlendi. Bis Schiff bazları (**6**), 200 ve 330 µM'ın altındaki IC_{50} değerleriyle daha iyi aktiviteye sahipti. Bileşikler DPPH ve FRAP testlerinin her ikisinde de orta ve yüksek seviyede antioksidan aktivite gösterdi. DPPH radikal temizleme testinde bileşiklerin bazılarının SC_{50} değerleri standart antioksidanlarınkine yakın 0,01 mg/mL seviyesinde olduğu görüldü. Benzer şekilde 8197 mM TEAC değeriyle yüksek FRAP aktivitesi tespit edildi. Yeni bileşiklerin yapısal farklılıkları antioksidan aktivitelerinde etkili oldu. Benzoilhidrazonlar (**2**), oksadiazoller (**3**) ve amino-triazoller (**4**), **5**-**8** billeşiklerine kıyasla yüksek aktivite gösterdiler.

1. Introduction

1,2,4-Triazoles and their derivatives are among the most studied heterocyclic compound classes in the field of medicinal and pharmaceutical chemistry [1]. Many heterocyclic compounds containing 1,2,4-triazole ring have various pharmacological properties such as anticonvulsant [2], antifungal [3], antimicrobial [4], antihypertensive [5], analgesic [6], antiviral [7], antiinflammatory [8], antioxidant [9,10], antitumor [11,12] and anti-HIV [13] activities.It is known that some heterocyclic compounds bearing 1,2,4-triazole ring are used as drugs. For example, fluconazole [14], itraconazole [15], ravuconazole [16], voriconazole [17] and posaconazole [18] are used as antifungal drugs in medicine. Furthermore, vorozole, letrozole and anastrozole are very effective aromatase inhibitors and are used in the treatment of breast cancer [19]. In addition, it is known that certain Schiff base derivatives bearing 1,2,4-triazole ring have important pharmacological properties [20-24]. Apart from the pharmacological use of Schiff bases, these compounds have been reported to exhibit fluorescence properties on some aqueous cationic solutions to be used in analytical applications [25-28]. In view of the facts mentioned, it is aimed to investigate both the fluorescence properties, antioxidant activities and carbonic anhydrase inhibitory potentials of newly synthesized 1,2,4-triazole compounds.

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2. Experimental

2.1. Synthesis

2.1.1. Materials and methods

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer. Chemical shifts are given as δ value in ppm and referenced to tetramethylsilane (TMS) as internal standard. The IR spectra were recorded by using potassium bromide pellets in a Perkin-Elmer 1600 series FTIR spectrometer. Elemental analyses were performed on a ECS 4010 Elemental Combustion System. For fluorescence measurements, a Photon Technologies International Quanta Master Spectrofluorimeter (model QM-4/2006) was used. The necessary chemicals were purchased from Merck and Fluka companies. Compounds **1a**,**b** and 1,2-bis (*o*-formylphenoxy) ethane were synthesized using published method [29].

2.1.2. Synthesis of hydrazones (2)

25 mL of absolute ethanol solution of benzhydrazide (0.01 mol) was added to 25 mL of absolute ethanol solution of iminoester hydrochloride (0.01 mol). The mixture was stirred for 12 h at 0-5 °C temperature. At the end of the reaction, the mixture was poured into iced water. The resulting precipitate was filtered and washed with cold water, then dried in vacuo. The resulting solid (**2a**,**b**) was used without further crystallization for the next step. The filtrate was evaporated under reduced pressure and dried in vacuo. The solid resude was recrystallized from water to give compounds **2a**,**b**.

2.1.2.1. Ethyl propanoate benzoylhydrazone (2a): Yield 50%, m.p. 80-81 °C; IR (KBr) ν (cm^{-1}): 3226 (NH), 1640 (C=O), 1618 (C=N), 799, 696 (arom. Ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 1.01 (t, 3H, J = 3.60 Hz, CH_3), 1.25 (t, 3H, J = 3.80 Hz, $OCH_2C\underline{H}_3$), 2.34 (q, 2H, J = 3.60 Hz, CH_2), 4.13 (q, 2H, J = 3.80 Hz, OCH_2), Arom. H: [7.44-7.47 (m, 3H), 7.78-7.81 (m, 2H)], 10.50 (s, 1H, NH). ^{13}C NMR (DMSO-d$_6$), δ (ppm): 11.10 (CH_3), 14.82 (CH_3), 18.12 (CH_2), 60.33 (OCH_2), Arom. C: [126.12 (2C), 127.11 (2C), 130.24, 134.10], 161.66 (C=N), 169.18 (C=O). Anal. Calcd for $C_{12}H_{16}N_2O_2$ (220.27): C, 65.43; H, 7.32; N, 12.72. Found: C, 65.39; H, 7.31; N, 12.78.

2.1.2.2. Ethyl cylopropanoate benzoylhydrazone (2b): Yield 55%, m.p. 98-99 °C; IR (KBr) ν (cm^{-1}): 3195 (NH), 1654 (C=O), 1627 (C=N), 721, 695 (arom. Ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 0.85 (d, 4H, cyclopropyl CH_2), 1.94 (m, 1H, cyclopropyl CH), 1.22 (t, 3H, J = 3.80 Hz, CH_3), 4.08 (q, 2H, J = 3.80 Hz, OCH_2), Arom. H: [7.40-7.54 (m, 3H), 7.82-7.87 (m, 2H)], 10.63 (s, 1H, NH). ^{13}C NMR (DMSO-d$_6$), δ (ppm): 6.30 (2 cyclopropyl CH_2), 8.25 (cyclopropyl CH), 13.91 (CH_3), 61.62 (OCH_2), Arom. C: [127.14 (2C), 128.11 (2C), 130.84, 134.02], 162.63 (C=N), 169.00 (C=O). Anal. Calcd for $C_{13}H_{16}N_2O_2$ (232.28): C, 67.22; H, 6.94; N, 12.06. Found: C, 67.20; H, 6.90; N, 12.10.

2.1.2.3. 2-Ethyl-5-phenyl-1,3,4-oxadiazole (3a): Yield 25%, m.p. 105-106 °C (lit. [30]: 104-105 °C); IR (KBr) ν (cm^{-1}): 1575 (C=N); 1063 (C-O), 793, 690 (arom. Ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 1.15 (t, J = 6.50 Hz, 3H, CH_3), 2.30 (q, 2H, J = 6.50 Hz CH_2), 7.34-7.84 (m, 5H, Ar-H).

2.1.2.4. 2-Cylopropy-5-phenyl-1,3,4-oxadiazole (3b): Yield 30%, m.p. 49-50 °C (lit. [31]: pale oil); IR (KBr) ν (cm^{-1}): 1611,1575 (C=N); 1068 (C-O), 792, 698 (arom. Ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 1.15-1.26 (m, 4H, cyclopropyl CH_2); 2.15-2.25 (m, 1H, cyclopropyl CH),7.43-8.00 (m, 5H, Ar-H).

2.1.3. Synthesis of amino compounds (4)

Hydrazine hydrate (0.01 mol, 99.99 %, 0.49 mL) was added to the solution of Compound **2a**,**b** in 50 mL of 1-propanol, and the mixture was refluxed for 24 hours under reflux. The reaction mixture was allowed to stand in the refrigerator for 1 night, after which a precipitate formed. The mixture was filtered, dried and purified by crystallization from 1-propanol. The resulting compound was identified as **4a**,**b**.

2.1.3.1. 3-Ethyl-5-phenyl-4-amino-4H-1,2,4-triazole (4a): Yield 87%, m.p. 132-133 °C; IR (KBr) ν (cm^{-1}): 3232, 3122 (NH_2), 1654, 1577 (C=N), 774, 689 (arom. Ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 1.28 (t, 3H, J = 3.60 Hz, CH_3), 2.75 (q, 2H, J = 3.80 Hz, CH_2), 6.05 (s, 2H, NH_2), Arom. H: [7.43-7.49 (m, 3H), 7.98-8.03 (m, 2H)]. ^{13}C NMR (DMSO-d$_6$), δ (ppm): 11.21 (CH_3), 17.19 (CH_2); Arom. C: [127.47, 127.76 (2C), 128.26 (2C), 129.12], 157.08 (triazole C-3), 152.36 (triazole C-5). Anal. Calcd for $C_{10}H_{12}N_4$ (188.23): C, 63.81; H, 6.43; N, 29.76. Found: C, 63.85; H, 6.45; N, 29.56.

2.1.3.2. 3-Cylopropyl-5-phenyl-4-amino-4H-1,2,4-triazole (4b): Yield 90%, m.p. 147-148 °C; IR (KBr) ν (cm^{-1}): 3275, 3211 (NH_2), 1628, 1575 (C=N); 763, 687 (arom. Ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 0.98 (d, 4H, cyclopropyl CH_2), 2.15 (m, 1H, cyclopropyl CH), 6.15 (s, 2H, NH_2), Arom. H: [7.40-7.49 (m, 3H), 7.96-8.00 (m, 2H)]. ^{13}C NMR (DMSO-d$_6$), δ (ppm): 4.69 (2 cyclopropyl CH_2), 7.35 (cyclopropyl CH), Arom. C: [127.36, 127.84 (2C), 128.31 (2C), 131.06], 157.64 (triazole C-3), 152.45 (triazole C-5). Anal. Calcd for $C_{11}H_{12}N_4$ (200.24): C, 65.98; H, 6.04; N, 27.98. Found: C, 66.04; H, 6.04; N, 27.87.

2.1.4. Synthesis of mono Schiff bases (5)

Compound **4a**,**b** (0.01 mol) was dissolved in 25 mL of acetic acid and 2,4-dichlorobenzaldehyde (0.01 mol) was added thereto and refluxed for 4 hours. At the end of this run, the reaction mixture was poured into iced water in a beaker. The precipitated product was filtered off and washed with water. The obtained solid was recrystallized from ethanol to afford the desired compound **5**.

2.1.4.1. 3-Ethyl-5-phenyl-4-(2,4-dichlorobenzylidenamino)-4H-1,2,4-triazole (5a): Yield 87%, m.p. 154-155 °C; IR (KBr) ν (cm^{-1}): 1584, 1550 (C=N), 868, 827, 775 (arom. ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 1.33 (t, 3H, J = 4.40 Hz, CH_3), 2.84 (q, 2H, J = 4.40 Hz, CH_2); Arom. H: [7.47-7.50 (m, 5H), 8.00-8.06 (m, 3H)], 8.84 (s, 1H, N=CH). ^{13}C NMR (DMSO-d$_6$), δ (ppm): 12.14 (CH_3), 19.24 (CH_2), Arom. C: [126.32, 127.28, 127.66, 128.12 (2C), 128.86 (2C), 129.10, 129.84, 134.46, 137.48, 143.12], 150.18 (triazole C-3), 151.16 (triazole C-5), 160.42 (C=N). Anal. Calcd for $C_{17}H_{14}Cl_2N_4$ (345.23): C, 59.15; H, 4.09; N, 16.23. Found: C, 59.6; H, 4.14; N, 16.27.

2.1.4.2. 3-Cylopropyl-5-phenyl-4-(2,4-dichlorobenzylidenamino)-4H-1,2,4-triazole (5b): Yield 92%, m.p. 165-166 °C; IR (KBr) ν (cm^{-1}): 1584, 1555 (C=N), 887, 777, 704 (arom. ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 1.07 (d, 4H, J = 6.80 Hz, 2 cyclopropyl CH_2), 2.10 (m, 1H, cyclopropyl CH), Arom. H: [7.47-8.11 (m, 8H, arom. H)], 8.72 (s, 1H, N=CH). ^{13}C NMR (DMSO-d$_6$), δ (ppm): 6.00 (2 cyclopropyl CH_2), 7.18 (cyclopropyl CH), Arom. C: [126.64, 127.52, 127.87, 128.09 (2C), 128.59 (2C), 129.15, 129.60, 135,81, 137,94, 142.39], 151.86 (triazole C-3), 150.14 (triazole C-5), 160.33 (C=N). Anal. Calcd for $C_{18}H_{14}Cl_2N_4$ (357.24): C, 60.52; H, 3.95; N, 15.68. Found: C, 60.50; H, 4.01; N, 15.72.

2.1.5. Synthesis of bis Schiff bases (6)

The solution of corresponding compound **4** (0.01 mol) in acetic acid was refluxed with 1,2-bis (*o*-formylphenoxy) ethane for 16 h. Then, the reaction mixture was added to ice-water while stirring. The precipitated product was filtered off and washed with water. The obtained solid was recrystallized from ethanol-water (1:2) to afford the desired compound **6**.

2.1.5.1. 1,2-Bis[o-(N-methylidenamino-3-ethyl-5-phenyl-4H -1,2,4-triazole-4-yl)-phenoxy]ethane (6a): Yield 78%, m.p. 210-211 °C; IR (KBr) ν (cm^{-1}): 1596, 1570 (C=N), 1252 (C-O), 772, 754, 697 (arom. ring). ^{1}H NMR (DMSO-d$_6$), δ (ppm): 1.05 (t, 6H, J = 3.80 Hz, 2CH_3),

2.53 (q, 4H, J = 3.80 Hz, $2CH_2$), 4.31 (s, 4H, $2OCH_2$), Arom. H: [6.97-7.01 (m, 2H), 7.04-7.16 (m, 2H), 7.21-7.33 (m, 6H), 7.50-7.61 (m, 6H), 7.86-7.90 (m, 2H)]; 8.53 (s, 2H, 2N=CH). ^{13}C NMR (DMSO-d_6), δ (ppm): 10.70 ($2CH_3$); 17.93 ($2CH_2$), 67.46 ($2OCH_2$), Arom. C: [113.53 (2C), 119.78 (2C), 121.24 (2C), 125.86 (2C), 126.66 (2C), 127.66 (2C), 128.46 (4C), 129.31 (2C), 134.67 (4C), 148.85 (2C)], 158.63 (2C, triazole C-3), 158.42 (2C, triazole C-5), 162.52 (2C, 2C=N). Anal. Calcd for $C_{36}H_{34}N_8O_2$ (610.72): C, 70.80; H, 5.61; N, 18.35. Found: C, 70.86; H, 5.55; N, 18.42.

2.1.5.2. 1,2-Bis[o-(N-methylidenamino-3-cylopropyl-5-phenyl-4H-1,2,4-triazole-4-yl)-phenoxy]ethane (6b): Yield 85%, m.p. 215-218 °C; IR (KBr) ν (cm^{-1}): 1598, 1576 (C=N), 1250 (C-O), 770, 760, 700 (arom. ring). ^{1}H NMR (DMSO-d_6), δ (ppm): 0.95 (d, 8H, J = 6.00 Hz, cyclopropyl CH_2), 2.70 (m, 2H, J = 6.80 Hz, cyclopropyl CH), 3.97 (s, 4H, $2OCH_2$), Arom. H: [6.97-7.05 (m, 2H), 7.31-7.36 (m, 8H), 7.67-7.71 (m, 6H), 7.90-7.94 (m, 2H)], 8.23 (s, 2H, 2N=CH). ^{13}C NMR (DMSO-d_6), δ (ppm): 5.78 (4C, cyclopropyl CH_2), 8.07 (2C, cyclopropyl CH), 66.89 (2C, $2OCH_2$), Arom. C: [113.48 (2C), 119.39 (2C), 121.35 (2C), 126.23 (2C), 126.91 (2C), 128.12 (2C), 128.58 (4C), 129.57 (2C), 134.77 (4C), 148.58 (2C)], 158.49 (2C, triazole C-3), 158.96 (2C, triazole C-5), 164.45 (2C, 2C=N). Anal. Calcd for $C_{38}H_{34}N_8O_2$ (634.74): C, 71.91; H, 5.40; N, 17.65. Found: C, 71.65; H, 5.32; N, 16.86.

2.1.6. Synthesis of Reduced Compounds 7 and 8

$NaBH_4$ (0.01 mol) was added in small portions over the solution of corresponding compound (**5** or **6**) (0.005 mol) dissolved in 50 mL dried methanol. Following a reflux for 30 min, the mixture was allowed to cool. After evaporation at 30-35 °C under reduced pressure, the solid residue was washed with cold water and dried in vacuo. The solid product was recrystallized from ethanol-water (1:2) to obtain the desired compound.

2.1.6.1. 3-Ethyl-5-phenyl-4-(2,4-dichlorobenzylamino)-4H-1,2,4-triazole (7a): Yield 97%, m.p. 120-121°C; IR (KBr) ν (cm^{-1}): 3211 (NH), 1643, 1590 (C=N), 862, 768, 691 (arom. ring). ^{1}H NMR (DMSO-d_6), δ (ppm): 1.22 (t, 3H, J = 3.60 Hz, CH_3), 2.67 (q, 2H, J = 3.60 Hz, CH_2), 3.97 (d, 2H, J = 8.00 Hz, NH-C<u>H</u>$_2$), Arom. H: [7.00-7.23 (m, 3H), 7.36-7.44 (m, 3H), 7.75-7.80 (m, 2H)], 7.13 (t, 1H, J = 8.00 Hz, N<u>H</u>-CH_2). ^{13}C NMR (DMSO-d_6), δ (ppm): 11.16 (CH_3), 17.03 (CH_2), 51.20 (NH-<u>C</u>H_2), Arom.-C: [127.04; 127.43 (2C); 127.95; 128.16 (2C); 128.50; 129.21; 132.57, 132.87, 133.26, 134.52], 151.86 (triazole C-3), 150.14 (triazole C-5), 160.33 (C=N). Anal. Calcd for $C_{17}H_{16}Cl_2N_4$ (347.25): C, 58.80; H, 4.64; N, 16.13. Found: C, 58.85; H, 4.60; N, 16.21.

2.1.6.2. 3-Cylopropyl-5-phenyl-4-(2,4-dichlorobenzylamino) -4H-1,2,4-triazole (7b): Yield 98%, m.p. 133-134 °C; IR (KBr) ν (cm^{-1}): 3213 (NH), 1670, 1640 (C=N), 863, 767, 690 (arom. ring). ^{1}H NMR (DMSO-d_6), δ (ppm): 0.96 (d, 2H, J = 6.80 Hz, cyclopropyl $2CH_2$), 2.18 (m, 1H cyclopropyl CH), 4.45 (d, 2H, J = 8.00 Hz, NH-C<u>H</u>$_2$), Arom. H: [6.34-6.66 (m, 2H), 7.45-7.56 (m, 4H), 7.98-8.04 (m, 2H)], 7.40 (t, 2H, J = 8.00 Hz, N<u>H</u>-CH_2). ^{13}C NMR (DMSO-d_6), δ (ppm): 4.71 (cyclopropyl $2CH_2$), 7.63 (cyclopropyl CH), 51.23 (NH-<u>C</u>H_2), Arom. C: [126.91, 127.34 (2C), 127.97 (2C), 128.35, 129.03, 130.54, 132.51, 133.14, 134.91, 142.42], 156.79 (triazole C-3), 151.91 (triazole C-5); Anal. Calcd for $C_{18}H_{16}Cl_2N_4$ (359.26): C, 60.18; H, 4.49; N, 15.60. Found: C, 60.24; H, 4.52; N, 15.56.

2.1.6.3. 1,2-Bis[o-(N-methylamino-3-ethyl-5-phenyl-4H-1,2,4-triazole-4-yl)-phenoxy]ethane (8a): Yield 92%, m.p. 225-226 °C; IR (KBr) ν (cm^{-1}): 3212 (NH), 1608, 1586 (C=N), 1256 (C-O), 772, 766, 690 (arom. ring). ^{1}H NMR (DMSO-d_6), δ (ppm): 1.01 (t, 6H, J = 3.60 Hz, $2CH_3$), 2.51 (q, 4H, J = 6.80 Hz, $2CH_2$), 3.84 (bs, 4H, 2NH-C<u>H</u>$_2$), 4.16 (s, 4H, $2OCH_2$), 6.74 (bs, 2H, 2N<u>H</u>-CH_2), Arom. H: [6.82-7.06 (m, 6H), 7.22-7.37 (m, 8H), 7.87-7.90 (m, 4H)]. ^{13}C NMR (DMSO-d_6), δ (ppm): 10.77 ($2CH_3$), 16.81 ($2CH_2$), 49.42 (2NH-<u>C</u>H_2), 66.16 ($2OCH_2$), Arom.-C: [111.44 (2C), 120.38 (2C), 123.90 (2C), 127.11 (4C), 127.26 (2C), 128.16 (4C), 129.08 (2C), 129.21 (2C), 129.73 (2C), 130.65 (2C)], 156.49 (2C, triazole C-3), 151.43 (2C triazole C-5), Anal. Calcd for $C_{36}H_{38}N_8O_2$ (614.75): C, 70.34; H, 6.23; N, 18.23. Found: C, 69.84; H, 6.64; N, 17.92.

2.1.6.4. 1,2-Bis[o-(N-methylamino-3-cylopropyl-5-phenyl-4H-1,2,4-triazole-4-yl)-phenoxy] ethane (8b): Yield 95%, m.p. 236-237 °C; IR (KBr) ν (cm^{-1}): 3241 (NH), 1600, 1587 (C=N), 1243 (C-O), 770, 756, 692 (arom. ring). ^{1}H NMR (DMSO-d_6), δ (ppm): 1.20 (d, 8H, J = 6.80 Hz, cyclopropyl CH_2), 2.85 (m, 2H, cyclopropyl CH), 3.63 (bs, 4H, 2NH-C<u>H</u>$_2$), 3.86 (s, 4H, $2OCH_2$), Arom. H: [6.66-6.75 (m, 4H), 7.08-7.18 (m, 2H), 7.36-7.48 (m, 8H), 7.80-7.94 (m, 4H)], 6.93 (bs, 2H, 2N<u>H</u>-CH_2). ^{13}C NMR (DMSO-d_6), δ (ppm): 6.13 (4C, cyclopropyl CH_2), 8.18 (2C, cyclopropyl CH), 48.79 (2C, 2NH-<u>C</u>H_2), 65.97 (2C, $2OCH_2$), Arom. C: [111.48 (2C), 120.07 (2C), 123.40 (2C), 126.92 (2C), 127.64 (2C), 128.22 (2C), 128.98 (4C), 129.42 (2C), 129.96 (4C), 130.12 (2C)], 156.15 (2C, triazole C-3), 153.52 (2C, triazole C-5); Anal. Calcd for $C_{38}H_{38}N_8O_2$ (638.77): C, 71.45; H, 6.00; N, 7.54. Found: C, 70.85; H, 5.85; N, 7.12.

2.2. Fluorescence measurements

To determine fluorescence properties of newly synthesized triazole compounds, their solubility was tested in different organic solvents. It was concluded that all of them were soluble in methanol except for **6a** and **8b**. Compound **6a** was soluble in DMSO:methanol (1:1), while **8b** in DMSO. The concentrations of all solutions were $4x10^{-5}$ M. Fluorescence spectra of the solutions were recorded between 360 nm and 560 nm for **5a**, **5b**, **6b**, **7a**, **7b**, and **8a** exciting at 350 nm. Excitation wavelength was 380 nm for **8b**. A 1-cm quartz cell was used in fluorescence measurements. The slit width was 1.0 nm.

2.3. Biological activity

The synthesized compounds were tested for antioxidant activity by utilizing the two frequently used methods: DPPH radical scavenging activity [32] and Ferric Reducing Antioxidant Power (FRAP) determinations [33]. The compounds were also tested for inhibitory activity against bovine carbonic anhydrase (BCA) enzyme by using esterase activity determination. The measurements were repeated in triplicates, and averages were used for activity calculations.

2.3.1. DPPH scavenging test

DPPH radical scavenging assay method is based on the measurement of the decrease in absorbance of DPPH solution at 517 nm because of scavenging of DPPH present when mixed with the test sample and incubated [32]. The sample solutions (750 µL) of varying concentration were mixed with equal volume of 50 µM DPPH solution, vortexed and incubated for 50 min to allow the scavenging to complete. The absorbance at 517 nm was measured and plotted against sample concentration to calculate SC_{50} value, the sample concentration providing 50% scavenging, i.e. half of the maximum absorbance achieved at zero sample concentration. Smaller SC_{50} values indicate better radical scavenging activity. The results are also expressed as %scavenging. BHT, Trolox and gallic acid were used as standard antioxidants for comparison.

2.3.2 Ferric reducing / antioxidant power (FRAP) test

The synthesized compounds were also tested for their antioxidant activity by using another commonly utilized method: FRAP assay. The assay method was developed by Benzie and Strain, with some modifications of the original method [33]. Working FRAP (Ferric reducing/antioxidant power) solution was prepared by mixing TPTZ solution (10 mM dissolved in 100 mM HCl) with 20 mM aqueous $FeCl_3$ solution and 300 mM acetate buffer (pH 3.6) in the ratio of 1:1:10, respectively. 50 µL of sample solution was mixed with 1.5 mL of freshly prepared FRAP reagent, and the mixture was vortexed. Following incubation at room temperature for 20 min, absorbance at 595 nm was determined against water. The activities of the compounds were expressed as micromolar TEAC, (Trolox Equivalent Antioxidant Capacity), which was obtained by using Trolox in the

same test (1000 - 500- 250- 125- 62,5 µM) to draw a calibration graph. Larger values of TEAC obtained show better activity.

2.3.3 Carbonic anhydrase inhibitory activity determination

The synthesized compounds were tested for carbonic anhydrase (CA) inhibitory (CAI) activity by using bovine enzyme (BCA) [34]. CA enzyme hydrolyzes *p*-nitrophenyl acetate (PNPA) to *p*-nitrophenol and *p*-nitrophenolate ions. The absorbance at the end of this reaction is measured at 348 nm resulting from *p*-nitrophenol and *p*-nitrophenolate formation. 300 µL of enzyme, 100 µL of pure water, 1100 µL of 0.05 M Tris-SO_4 (pH: 7.4) and 1500 µL of 3 mM *p*-nitrophenyl acetate substrate were added for esterase activity, and absorbance at 348 nm was measured as a function of time. Potential inhibitors decrease absorbance change as they prevent PNPA hydrolysis by inhibiting the enzyme. Inhibitory activity is expressed as IC_{50} values, the concentration of sample providing 50% inhibition of enzyme activity.

3. Results and discussion

3.1. Synthesis and characterization

In this study, we report a general and convenient method for the synthesis of unsymmetrical 3-ethyl/cylopropyl-5-phenyl-4H-1,2,4-triazole derivatives. The synthesis steps of the target compounds are shown in Scheme. Benzoylhydrazones **2a**,**b** were synthesized by the reactions of alkyl imidate hydrochlorides **1a**,**b** with benzhydrazide. 1,3,4-oxadiazoles **3a**,**b** were obtained in the same reaction media with compounds **2a**,**b**. Compounds **3a**,**b** are known [30, 31]. Compounds **4a,b** were obtained by the treatment of compounds **2a**,**b** with hydrazine hydrate. 3-Alkyl-5-phenyl-4-(2,4-dichlo-robenzyliden-amino)-4H-1,2,4-triazole (mono Schiff bases) **5a**,**b** were prepared by the condensation of 3-alkyl-5-phenyl-4-amino-4H-1,2,4-triazole **4a**,**b** with 2,4-dichlorobenzaldehyde in acetic acid at refluxing temperature for 4 h. 1,2-bis[o-(N-methylidenamino-3-alkyl-5-phenyl-4H-1,2,4-triazole-4-yl) phenoxy]ethane (bis Schiff bases) **6a**,**b** were prepared by the condensation of 3-alkyl-5-phenyl-4-amino-4H-1,2,4-triazole **4a**,**b** with 1,2-bis (*o*-formylphenoxy) ethane in acetic acid at refluxing temperature for 16 h. Then compounds **5a**,**b** and **6a**,**b** were converted to their reduced derivatives **7a**,**b** and **8a**,**b** by treating with sodium borohydride in methanol.

Because of imine character, the reduction of compounds **5a**,**b** and **6a**,**b** can be possible and should be taken into consideration [35-37]. The reduction of the 3-alkyl-5-phenyl-4H-1,2,4-triazole (**4a**,**b**) ring may also occur [38]. Hence, the attempts of the reduction of compounds **5a**,**b** and **6a**,**b** may result in the formation of various products. However in the study, the reduction of only the imino group of compounds **5a**,**b** and **6a,b** was achieved using $NaBH_4$ in methanol as a selective reducing agent.

The IR spectra of compounds **2a**,**b** showed absorption bands at about 3200, 1650 and 1620 cm^{-1} regions resulting from the NH, C=O and C=N functional groups, respectively. The FT-IR spectra of compounds **4** showed an absorption band at 3232, 3122 (for **4a**), 3275, 3211 (for **4b**) indicating the presence of NH_2 signals. In compounds **5a**,**b** and **6a**,**b** NH_2 bands were absent, and C=N absorption bands at about 1580, 1550 cm^{-1} were observed. Compounds **7a**,**b** and **8a**,**b** showed characteristic NH stretching bands at around 3210 cm^{-1}. In the ^{1}H NMR spectra of compounds **2a**,**b** characteristic OCH_2CH_3 signals appeared at around δ 1.15 ppm (t, 3H, CH_3) and δ 4.09 ppm (q, 2H, CH_2), and NH signals were recorded at around δ 10.50 ppm. Characteristic amino proton (NH_2) of compound **4a**,**b** were detected at about δ 6.10 ppm. The ^{1}H-NMR signals for -N=CH group of compounds **5a**,**b** were observed at δ 8.84 (for **5a**) and 8.72 (for **5b**). The ^{1}H-NMR signals for -N=CH and –OCH_2CH_2O- signals of compounds **6a**,**b** were observed at around δ 8.20 (s, 1H, NH) ppm and 4.00 (s, 4H, 2 OCH_2) ppm, respectively. Characteristic –NH-CH_2- signals of reduced compounds **7a**,**b** and **8a**,**b** were detected at about δ 7.26-7.58 (NH) and δ 4.03-4.43 ppm (-CH_2). In the ^{13}C NMR spectra of compounds **2a**,**b** characteristic C=O signals appeared at around δ 170 ppm. The ^{13}C NMR signals for triazole C-3 and triazole C-5 signal of compounds **4a**,**b** were observed at around δ 157 ppm and δ 152 ppm, respectively. The ^{13}C-NMR signals for the –N=CH- group of Schiff bases (**5a**,**b** and **6a**,**b**) were recorded at around δ 164 ppm. –NH-CH_2- carbon signal of reduced compounds (**7a**,**b** and **8a**,**b**) were recorded at around δ 48 ppm in the ^{13}C-NMR.

3.2. Fluorescence properties of the compounds

Fig. 1 shows the fluorescence spectra of the new compounds (**5-8**). Excitation wavelengths between 300-400 nm were tested to reach the highest fluorescence intensity. The maximum emission band was obtained at 395 nm when the methanolic solutions were excited at 350 nm except for **6a**. The maximum emission band was at 380 nm for **6a**. When excited at 380 nm, **8b** gave a maximum emission band at 435 nm. The reason of obtaining the emission maximum at different wavelengths is the use of different solvents. As seen from Fig. 1, the emission band intensities for **5a**, **5b**, **7a**, **7b** and **8a** are very low. This means that these compounds have low fluorescence properties. This is an expected result, because the compounds are small and have less π conjugation system except for **8a**. Since **8a** has lower π conjugation, its fluorescence property is lower with respect to **6a**. Compound **6a** is an imine compound and has chromophore C=N group. Probably, because of this reason **6a** shows high fluorescence in comparison to the amine compound **8a**. As expected, **6b** shows the highest fluorescence intensity with respect to dramatic emission enhancement among the compounds in methanol. This compound has two C=N groups and high π conjugation system. As seen from Fig. 1, there is a red shift in the spectra in DMSO of the amine compound **8b** with respect to other spectra in methanol. This can be explained with solvent effect.

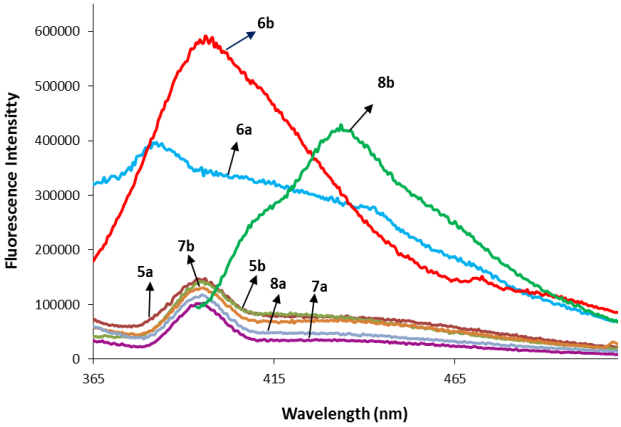


Fig. 1. Fluorescence spectra of **5-8**. Concentration: $4x10^{-5}$ M. Solvent: DMSO for **8b**, DMSO:methanol (1:10) for **6a**, and methanol for the others. Excitation wavelength is 380 nm for **8b** and 350 nm for other compounds.

3.3. Biological activity

Antioxidant activity is the most common biological activity studied for both synthesized compounds and the compounds or mixtures obtained from natural sources. Antioxidant compounds can be used in many different applications from material science to food industry. The compounds **2-8** were tested for antioxidant activity by DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) tests, the two most common antioxidant methods. The results of the two methods were also evaluated comparatively.

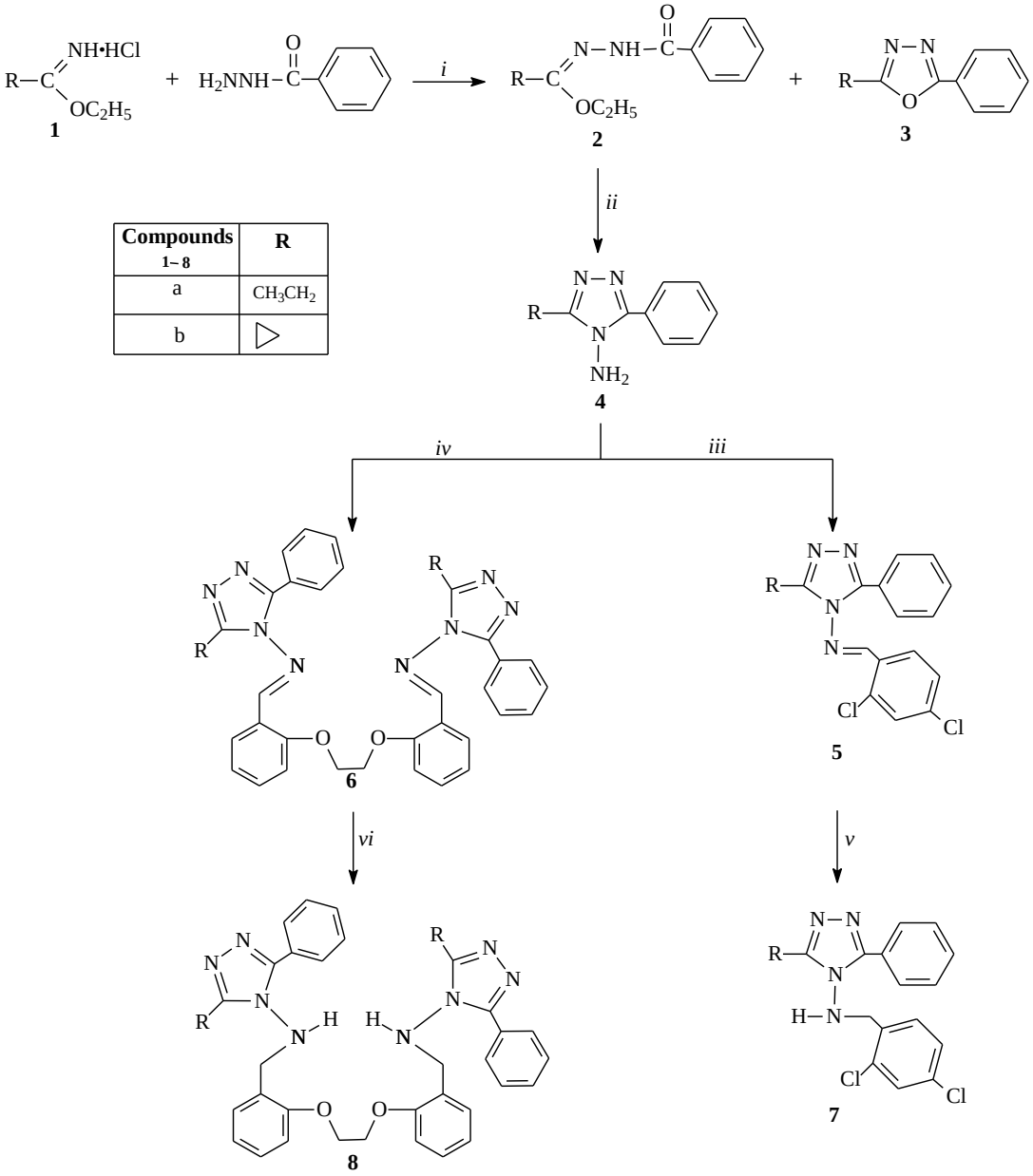
The compounds were also evaluated for their inhibitory performance against carbonic anhydrase enzyme by using esterase activity determination. CAI activity can be valuable in applications in medicinal therapy, diagnosis and environmental issues.

The biological activity tests were applied in triplicates. The statistical evaluation was done, and the results showed percentage relative standard deviation of 1.3-4.9%. Results are given as means of the measurements or as data obtained from linear regression analyses.

3.3.1 Antioxidant activities

DPPH scavenging test was used to determine antioxidant activity of the synthesized compounds. The results are expressed as SC_{50} values (Fig. 2a) and %DPPH scavenging at same concentrations of the samples (Fig. 2b). DPPH test results show that cyclopropyl substituted mono Schiff base (**5b**) had higher activity compared to cyclopropyl substituted bis Schiff base (**6b**) and their reduced forms (**7b** and **8b**), while reduced form of ethyl substituted bis Schiff base (**8a**) had higher activity compared to ethyl substituted other three compounds (**5a**,**6a** and **7a**). These findings hint to a structure activity relationship in the sense that not only the overall structure but also a small part of the compound may switch the order of activity.

A similar trend was observed with the FRAP test results (Fig. 3). The substituents appear to switch the order of activity going from compounds **5** to **8**. When the results of the two antioxidant tests are evaluated, it is clear that hydrazones (**2**), oxadiazoles (**3**) and amino compounds (**4**) are much more active than Schiff bases (**5** and **6**) and their reduced forms (**7** and **8**). Compounds **3a** and **4a** showed high DPPH scavenging performance with SC_{50} values of 0.0150 and 0.0160 mg/mL, respectively. A good correlation was achieved between DPPH scavenging and FRAP test results (Fig. 4).



Scheme. Reagents and conditions: i: Absolute ethanol, Stirring at 0-5 °C; ii. $NH_2NH_2.H_2O$, 1-propanol, reflux; iii. and iv. aldehyde, acetic acid, reflux v and vi. methanol, $NaBH_4$, reflux.

3.3.2 Carbonic anhydrase inhibitory (CAI) activity test

CAIs are valuable for their use in many medical conditions including glaucoma, epilepsy, and cancer. CAIs are used to lower intra ocular pressure (IOP) in glaucoma [39]. Cancer cells produce CA IX and XII isozymes whose activity is vital to tumor cell survival, and investigations for selective inhibitors of these enzymes have attracted much attention in the recent decade in the search of new leads [40]. Therefore, the synthesized compounds were tested for CAI by using bovine enzyme (BCA). Only six of the compounds showed inhibitory activity against BCA in 200 – 2500 µM range (Table). Bis Schiff bases (**6**) showed better inhibition with low IC_{50} values, and a further follow-up may be done with similar compounds.

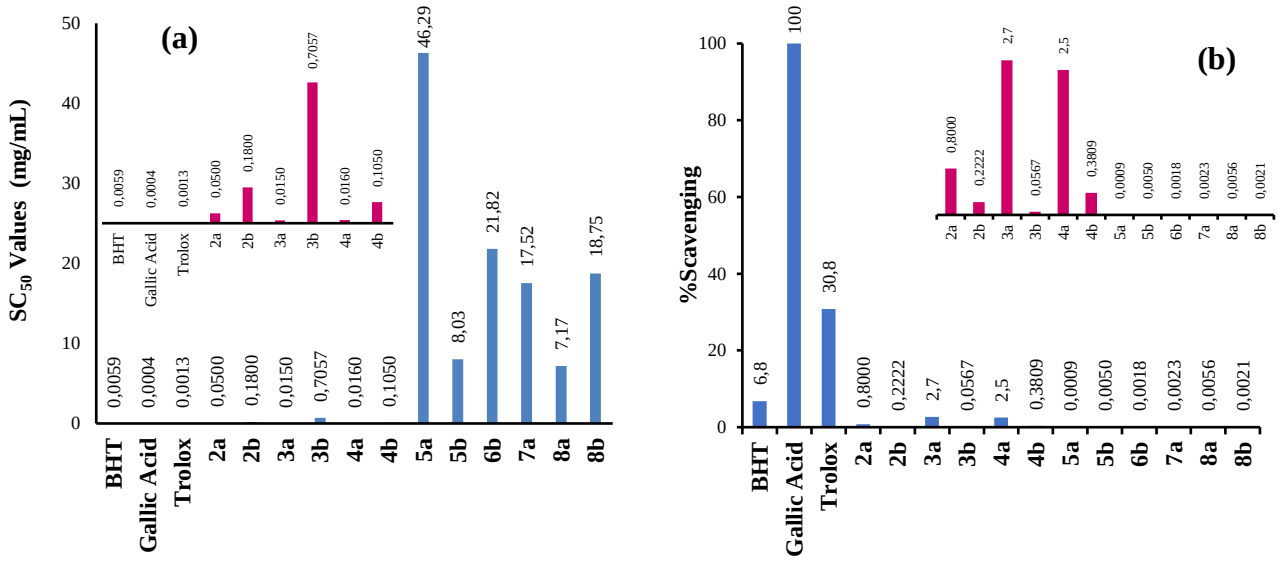


Fig. 2. (a) SC_{50} values of the compounds and standard antioxidants in DPPH scavenging test. Lower values represent better activity. **(b)** %DPPH scavenging of compounds and standards at 0.8 µg/mL final concentration. Compounds **6a** and **7b** were not active.

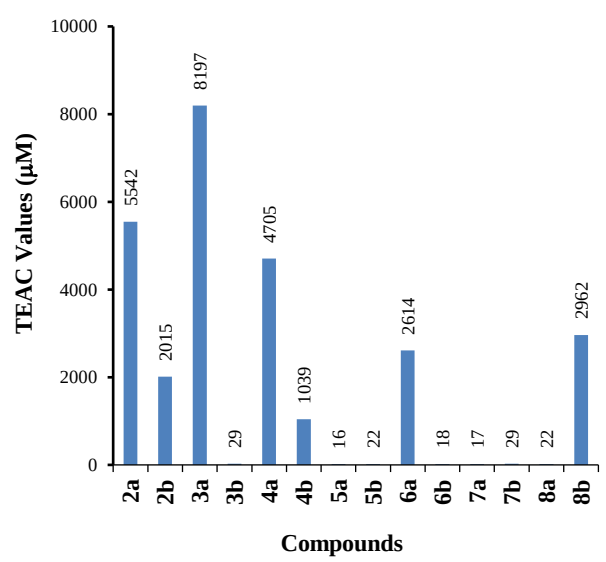


Fig. 3. Trolox Equivalent Antioxidant Capacity (TEAC) values of the compounds in FRAP test. Higher values represent better activity.

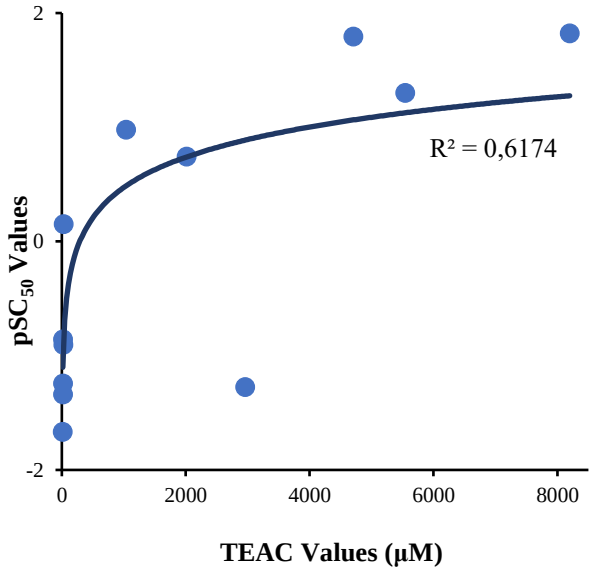


Fig. 4. Correlation between FRAP and DPPH tests constructed as a graph of TEAC values vs pSC_{50} values of the tested compounds. pSC_{50} values are – log of SC_{50} values.

4. Conclusion

New heterocyclic compounds containing 1,2,4-triazole ring were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and elemental analyses. The fluorescence properties of **5-8** compounds were determined and **6b** shows the highest fluorescence intensity with respect to dramatic emission enhancement among the compounds in methanol. In addition, the newly synthesized compounds showed good antioxidant activities in DPPH scavenging and FRAP assays, and the results of the two tests correlated. Compounds **2-4** were much more active than compounds **5-8**. Bis Schiff bases (**6**) showed considerable CAI activity, which hints that further synthesis may be done with new triazoles with bis Schiff base structure.

Table. IC_{50} values in carbonic anhydrase inhibitory (CAI) activity test

Sample/Standard	IC_{50} Value (µM)
Sulfanilamide	4
Acetazolamide	0.12
5b	590
6a	200
6b	330
7a	1650
8a	2500
8b	250

The other compounds were inactive.

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