Synthesis and biological studies of thiol derivatives containing imidazole moiety

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A series of thiol derivatives containing imidazole moiety have been synthesized by cyclisation of ethyl acetoacetate with substituted aromatic amines in presence of potassium thiocyanate to form tetra substituted compounds which are oxidized in presence of selenium dioxide to afford diketo compound 2-(4-ethoxy-1-(4-hydroxyphenyl)-2-mercapto-1H-imidazol-5-yl)-2-oxoacetaldehyde 2. The compound 2 on condensation with different aromatic aldehydes forms thiol derivatives 3a-j. The structural elucidation of all the synthesized compounds have been confirmed by their spectral/elemental data analysis. All the synthesized thiol derivatives have been investigated for their antibacterial and antifungal activity and show moderate to very good activity.

Keywords: Ethyl acetoacetate, substituted aromatic amines, aromatic aldehydes, potassium thiocyanate, selenium dioxide, dioxane

Thiol is an organosulphur compound that contains a carbon bonded sulfhydryl (−C−SH or R−SH) group. These are sulphur analogues of alcohols and are often referred to as mercaptan1. This group plays a very important role in biology. Thiol derivatives are compounds which contain a functional group of sulphur atoms and hydrogen atom. Thiol derivatives containing imidazole moiety are potential bioactive agents due to their wide spectrum of pharmacological activities like antibacterial, fungicidal2, sympathominetic activity3, anti-retrovirus activity and pharmaceutical compositions effective for the treatment of retrovirus infection such as human immunodeficiency syndromes4-7. Thiol derivatives either oral or nebulized have shown benefit in respiratory diseases also. Their mode of action is likely to differ accordingly to route of administration. There are several thiol derivatives and it is unclear which of these may be beneficial in cystic fibrosis8. This class of compounds provides an outstanding case history of modern drug development and also emphasizes the unpredictability of biological activity from structural modification of prototype drug molecule.

Considering the huge demand of tri and tetra substituted imidazole derivatives in the pharmaceuticals9, these diverse properties of imidazole-2-thiol and their derivatives have lead to the synthesis of these compounds in order to study their biological activities. In continuation of our research work, we used simple method to form thiol adducts which on condensation with aromatic aldehydes afforded thiol derivatives containing imidazole moiety. All the synthesized thiol derivatives were characterized by using elemental analysis, IR, 1H and 13C NMR spectral studies.

Results and Discussion

The reaction was completed in three steps. In the first step, thiol derivatives formed 1-(4-ethoxy-1-(4-hydroxyphenyl)-2-mercapto-1H-imidazol-5-yl)ethanone 1, which on oxidation with selenium dioxide converted into the diketo compound 2-(4-ethoxy-1-(4-hydroxyphenyl)-2-mercapto-1H-imidazol-5-yl)-2-oxoacetaldehyde 2. Thereafter, the compound 2 was reacted with substituted aromatic aldehydes in presence of glacial acetic acid and ammonium acetate to form the desired products 3a-j (Table I). All the step of reactions are nicely summarized in Scheme I.

Experimental Section

All chemicals and solvents used in this study were purchased locally from Aldrich (Germany) and Merck (Germany). Melting points are uncorrected and were determined in open capillary tubes. Progress of the reaction and homogeneity of the compounds were monitored by thin layer chromatography (TLC) which...
was performed using silica gel G (Merck) and compounds were visualized using iodine chamber. All the compounds were routinely checked by IR, $^1$H and $^{13}$C NMR and mass spectrometry. IR spectra were recorded in KBr pellets using Perkin-Elmer model 2000 spectrometer and reported wave numbers are given in cm$^{-1}$. $^1$H and $^{13}$C NMR spectra were recorded on Bruker Avance 300 MHz and 75 MHz spectrometers respectively, using CDCl$_3$/DMSO-$d_6$ as solvent and Tri Methyl Silane (TMS) as an internal standard. Chemical shift (δ) are given in parts per million (ppm). The splitting patterns are reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad singlet (bs). Coupling constants are given in Hz. Mass spectra were obtained on a JMS-T100LC, Accu TOF mass spectrometer (DART). The elemental analysis (CHNS) of compounds was carried out on a Carlo-Erba 1108 elemental analyzer. Their results were found to be in good agreement with the calculated values.

**Procedure for the synthesis of thiol derivatives**

Ethyl acetoacetate (1 mmol) was added drop-wise to the solution of substituted aromatic amines (1 mmol) in ethanol (20 mL) with constant stirring for 1.0 h at 0-5°C. After the addition, the reaction mixture was stirred for 1.0 h at RT and left for 12 h more. Then potassium thiocynate (1 mmol) was added to the reaction mixture which was refluxed for 1.0 h, cooled and poured into crushed ice. The product which separated out was filtered, washed with water, dried and purified by recrystallization from ethanol to give 1-(4-ethoxy-1-(4-hydroxyphenyl)-2-mercapto-$^{1}$H-imidazol-5-yl)ethanone 1. Homogeneity of the compound was confirmed by TLC (Toluene: Methanol, 8:2). Brown solid compound. Yield 85%. m.p.154°C. IR(KBr): 1611 (C=O), 1481 (C=N), 1444 (C=C), 1237 (C-O), 3460 (Ar-OH), 2560 cm$^{-1}$ (S-H); $^1$H NMR (300 MHz, DMSO-$d_6$): δ 2.37 (s, 3H, COCH$_3$), 3.99 (2H, q, OCH$_2$), 1.29 (t, 3H, CH$_2$CH$_3$), 9.91 (s, 1H, Ar-OH), 6.99-8.24 (m, 4H, $p$-hydroxy...
Spectral data of thiol derivatives

4-(5′-Ethoxy-2′-mercapto-2-phenyl-1H,3′H-4,4′-biimidazol-3′-yl)phenol, 3a: IR (KBr): 3442 (N-H), 3025 (C-H), 1570 (C=N), 1455 (C=C), 1320 (C-N), 2555 (S-H), 1552 (C=N), 1440 (C=C), 1230 (C-O), 692 cm⁻¹ (C-S); ¹H NMR (300 MHz, DMSO-d₆): δ 7.40-7.55 (m, 4H, Ar-H), 7.35-8.10 (m, 4H, Ar-H), 7.87 (d, 1H, C-H, imidazole), 10.30 (bs, 1H, Ar-OH), 140.7, 132.8, 130.4, 134.5, 129.6, 119.3, 116.4, 64.6, 14.8; MS: m/z 378.54 (M⁺).

3b: IR (KBr): 3436 (N-H), 3025 (C-H), 1555 (C=N), 1455 (C=C), 1230 (C-N), 2555 (S-H), 1552 (C=N), 1440 (C=C), 1230 (C-O), 692 cm⁻¹ (C-S); ¹H NMR (300 MHz, DMSO-d₆): δ 7.40-7.55 (m, 4H, Ar-H), 7.35-8.10 (m, 4H, Ar-H), 7.87 (d, 1H, C-H, imidazole), 10.30 (bs, 1H, Ar-OH), 140.7, 132.8, 130.4, 134.5, 129.6, 119.3, 116.4, 64.6, 14.8; MS: m/z 378.54 (M⁺).

Table I — Physical and analytical characterization data of compounds 3a - j

<table>
<thead>
<tr>
<th>Compd</th>
<th>Mol. Formula</th>
<th>Substituent R</th>
<th>Mol. Wt</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
<th>Calcd (Found) %</th>
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<tr>
<td>3a</td>
<td>C₂₀H₁₈O₄N₂S</td>
<td>H</td>
<td>378</td>
<td>80</td>
<td>220-240</td>
<td>63.49 4.76 14.81 8.46</td>
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<tr>
<td>3b</td>
<td>C₂₀H₁₇O₄N₂SCl</td>
<td>Cl</td>
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<td>268</td>
<td>(63.43 4.71 14.79 8.41)</td>
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<tr>
<td>3c</td>
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<td>4-OH</td>
<td>394</td>
<td>82</td>
<td>252</td>
<td>60.01 4.56 14.21 8.12</td>
</tr>
<tr>
<td>3d</td>
<td>C₂₀H₁₇O₃N₂S</td>
<td>4-NO₂</td>
<td>423</td>
<td>88</td>
<td>272</td>
<td>(60.07 4.48 14.15 8.14)</td>
</tr>
<tr>
<td>3e</td>
<td>C₁₀H₁₀O₃N₂S</td>
<td>4-OCH₃</td>
<td>408</td>
<td>84</td>
<td>260</td>
<td>56.73 4.01 16.54 7.56</td>
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<tr>
<td>3f</td>
<td>C₂₀H₁₇O₃N₂SCl</td>
<td>Cl</td>
<td>412.5</td>
<td>84</td>
<td>262</td>
<td>(56.71 3.99 16.48 7.47)</td>
</tr>
<tr>
<td>3g</td>
<td>C₂₀H₁₈O₃N₂S</td>
<td>2-OH</td>
<td>394</td>
<td>86</td>
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<tr>
<td>3h</td>
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<td>3-NO₂</td>
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<td>84</td>
<td>278</td>
<td>(60.07 4.48 14.15 8.14)</td>
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<tr>
<td>3i</td>
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<td>4-C₃H₆N</td>
<td>421</td>
<td>84</td>
<td>264</td>
<td>62.70 5.46 16.62 7.60</td>
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<tr>
<td>3j</td>
<td>C₂₀H₁₇O₃N₂SF</td>
<td>2-F</td>
<td>396</td>
<td>76</td>
<td>270</td>
<td>(60.54 4.23 14.11 8.02)</td>
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</table>

The synthesized compound 1 (0.5 mmol) undergoes oxidation with selenium dioxide (0.5 mmol) according to the literature method¹⁰, to form product 2-(4-ethoxy-1-(4-hydroxyphenyl)-2-mercapto-1H-imidazol-3-yl)-2-oxoacetdehyde 2 in which COCH₃ functional group was converted into dicarbonyl group. Pale yellow solid compound. Yield 75%. m.p.162°C. ¹H NMR: δ 9.49 (s, 1H, CHO), 10.02 (s, 1H, OH); MS: m/z 292 (M⁺), 293 (M+1).

Table I — Physical and analytical characterization data of compounds 3a - j

Thereafter, the compound 2 (1 mmol) was reacted with substituted aromatic aldehyde (1 mmol) and ammonium acetate (2 mmol) in presence of glacial acetic acid in round bottom flask under reflux for 4-5 h. The reaction mixture was cooled to RT and the whole content was poured into cold water (200 mL). The precipitates so obtained were filtered, washed with water, dried and purified by recrystallization from acetone to get the desired products 3a-j.
(C-O), 3324 (Ar-OH), 656 cm\(^{-1}\) (C-S); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.90 (d, 1H, C-H, imidazole), 10.38 (bs, 1H, N-H, imidazole), 7.41-7.86 (m, 4H, Ar-H), 6.90-7.78 (m, 4H, \(p\)-hydroxy phenyl), 9.45 (s, 2H, 2\(x\)-Ar-H), 3.91 (q, 2H, OCH\(_2\)CH\(_3\)), 1.27 (t, 3H, OCH\(_2\)CH\(_3\)), 3.26 (s, 1H, S-H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 135, 125, 127, 126.8, 148.9, 116.4, 129.8, 122.7, 118.3, 146.9, 119.3, 140.7, 126.3, 130.9, 118.4, 154.5, 115.4, 131.5, 64.6, 14.8; MS: \(m/z\) 394.55(M\(^+\)).

4-\((5^\prime\)-Ethoxy-2^-mercapto-2-(4-nitrophenyl)-1\(H,3\(H\)-4,4^-biimidazol-3^-yl)phenol, 3d: IR (KBr): 3430 (N-H), 3024 (C-H), 1564 (C=\(N\)), 1426 (C=C), 1320 (C-N), 2558 (S-H), 1550 (C=\(N\)), 1426 (C=C), 1218 (C-O), 3310 (Ar-OH), 654 (C-S), 1360 (NO\(_2\)), 1343 cm\(^{-1}\) (C-N); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.95 (d, 1H, C-H, imidazole), 10.36 (bs, 1H, N-H, imidazole), 7.42-8.25 (m, 4H, Ar-H), 6.65-7.20 (m, 4H, \(p\)-hydroxy phenyl), 9.32 (s, 1H, Ar-OH), 3.96 (q, 2H, OCH\(_2\)CH\(_3\)), 1.24 (t, 3H, OCH\(_2\)CH\(_3\)), 3.24 (s, 1H, S-H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 135, 127, 126.8, 148.9, 116.4, 129.8, 122.7, 118.3, 146.9, 119.3, 140.7, 139.6, 126.5, 122.1, 145.9, 126.4, 64.6, 14.8; MS: \(m/z\) 423.24(M\(^+\)).

4-\((5^\prime\)-Ethoxy-2^-mercapto-2-(4-methoxyphenyl)-1\(H,3\(H\)-4,4^-biimidazol-3^-yl)phenol, 3e: IR (KBr): 3425 (N-H), 3015 (C-H), 1545 (C=\(N\)), 1426 (C=C), 1210 (C-O), 3345 (Ar-OH), 2561 (S-H), 1315 (C-N), 1430 (C=C), 689 cm\(^{-1}\) (C-S); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.83 (d, 1H, C-H, imidazole), 10.35 (bs, 1H, N-H, imidazole), 6.82-7.59 (m, 4H, Ar-H), 6.92-7.87 (m, 4H, \(p\)-hydroxy phenyl), 3.88 (s, 3H, OCH\(_3\)), 9.44 (s, 1H, Ar-OH), 3.82 (q, 2H, OCH\(_2\)CH\(_3\)), 1.25 (t, 3H, OCH\(_2\)CH\(_3\)), 3.28 (s, 1H, S-H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 135, 127, 126.8, 148.9, 116.4, 129.8, 122.7, 118.3, 146.9, 119.3, 140.7, 131.1, 123.4, 147.9, 124.1, 130.6, 133.5, 64.6, 14.8; MS: \(m/z\) 423.35(M\(^+\)).

4-\((2^-\)\(p\)-4,4^-biimidazol-3^-yl)phenol, 3i: IR (KBr): 3420 (N-H), 3010 (C-H), 1543 (C=\(N\)), 1415 (C=C), 1190 (C-O), 2555 (S-H), 1520 (C=\(N\)), 1450 (C=C), 675 cm\(^{-1}\) (C-S); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.78 (d, 1H, C-H, imidazole), 10.37 (bs, 1H, N-H, imidazole), 7.43-7.88 (m, 4H, Ar-H), 6.45-7.38 (m, 4H, \(p\)-hydroxy phenyl), 2.93 (s, 6H, N\(_2\)CH\(_2\)), 9.42 (s, 1H, Ar-OH), 3.87 (q, 2H, OCH\(_2\)CH\(_3\)), 1.25 (t, 3H, CH\(_2\)CH\(_3\)), 3.26 (s, 1H, S-H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 135, 127, 126.8, 148.9, 116.4, 129.8, 122.7, 118.3, 146.9, 119.3, 124.1, 130.6, 133.5, 64.6, 14.8; MS: \(m/z\) 421.48(M\(^+\)).

4-\((5^-\)\(2^-\)\(2\)-fluorophenyl)-2^-mercapto-1\(H,3\(H\)-4,4^-biimidazol-3^-yl)phenol, 3j: IR (KBr): 3433 (N-H), 3019 (C-H), 1545 (C=\(N\)), 1430 (C=C), 1327 (C-N), 1105 (C-F), 2539 (S-H), 1545 (C=C), 1440 (C=C), 1190 (C-O), 3310 (Ar-OH), 676 cm\(^{-1}\) (C-S); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.80 (d, 1H,
C-H, imidazole), 10.40 (bs, 1H, N-H, imidazole), 7.34-7.61 (m, 4H, Ar-H), 7.35-8.20 (m, 4H, p-hydroxy phenyl), 9.32 (s, 1H, Ar-OH), 3.79 (q, 2H, OCH₂CH₃), 1.20 (t, 3H, OCH₂CH₃), 3.28 (s, 1H, S-H); 13C NMR (75 MHz, DMSO-d₆): δ 135, 125, 127, 126.8, 148.9, 116.4, 129.8, 122.7, 118.3, 146.9, 119.3, 140.7, 130.5, 128.5, 120.6, 160.7, 116.1, 130.6, 64.6, 14.8; MS: m/z 396.40 (M⁺).

Biological Studies
Antibacterial and antifungal studies

All the compounds have been screened for their antibacterial and antifungal activities using cup plate agar diffusion method by measuring the inhibition zone in mm. Ofloxacin (100 µg/mL) was used as standard drug for antibacterial activity and voriconazole (100 µg/mL) as standard drug for antifungal activity. The compounds were screened for antibacterial activity against *E. coli*, *B. subtilis* and *S. aureus* in nutrient agar medium and for antifungal activity against *Candida albicans* in Sabouraud’s dextrose agar medium. The zone of inhibition observed around the cup after respective incubation was assumed and percent inhibition of the compounds was calculated. The results are presented in Table II. By visualizing inhibition zone it could be observed that compound 3j showed the highest activity (86.56%) against *S. aureus*, whereas compounds 3b, 3d, 3e showed the highest activity (70.12%) against *E. coli* when compared with standard drug ofloxacin. The rest of the compounds showed moderate to good activity against *E. coli*, *B. subtilis* and *S. aureus*. The thiol derivative 3i having 4-dimethyl amino phenyl group showed the highest antifungal activity (80.24%) against *C. albicans*. When this group was replaced by 4-hydroxy group in 3c, there was slight decrease of antifungal activity (63.55%). The compound 3h showed minimum activity. Thus, it can be concluded from the screening results that thiol derivatives were most effective against all microorganism at a concentration of 100 µg/mL.

Conclusion

The synthesis and biological activities of thiol derivatives has been described. It is worth mentioning that combination of two biologically active moieties, thiol and imidazole, profoundly influence the biological activity. The biological data revealed that with slight modifications in the structure one can plan for better drug design.

Acknowledgement

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References

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