**SUPPLEMENTARY MATERIAL**

**Unsymmetrical Thiourea Derivatives: Synthesis and Evaluation as Promising Antioxidant and Enzyme Inhibitors**

**Table-S1:** *In vitro* antioxidant, lipoxygenase, and xanthine inhibitory activities of thiourea derivatives **1-20**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Comp.** | **Structure** | **Antioxidant Activity** | **Lipoxygenase Inhibition Activity** | **Xanthine Oxidase Inhibition Activity** |
| **IC50 ± SEMa (*µ*M)** | | |
| **1** |  | 35.25 ± 0.55 | 29.6 ± 0.51 | 78.9 ± 0.11 |
| **2** |  | 26.42 ± 0.06 | 31.7 ± 0.28 | 20.6 ± 0.72 |
| **3** |  | 51.93 ± 0.47 | 49.8 ± 0.38 | 87.6 ± 0.38 |
| **4** |  | 76.1 ± 0.76 | >100 | 77.7 ± 0.38 |
| **5** |  | 80.2 ± 0.08 | >100 | 97.5 ± 0.40 |
| **6** |  | 69.8 ± 0.73 | >100 | 69.7 ± 0.11 |
| **7** |  | 87.3 ± 0.65 | >100 | 86.3 ± 0.84 |
| **8** |  | 79.9 ± 0.22 | >100 | 98.7 ± 0.22 |
| **9** |  | 22.2 ± 0.09 | 31.2 ± 0.93 | 31.6 ± 0.65 |
| **10** |  | 56.7 ± 0.11 | 46.6 ± 0.92 | 12.4 ± 0.83 |
| **11** |  | 56.7 ± 0.03 | 65.1 ± 0.11 | 26.8 ± 0.03 |
| **12** |  | 37.8 ± 0.87 | 44.4 ± 0.83 | 21.4 ± 0.93 |
| **13** |  | 87.6 ± 0.28 | 89.6 ± 0.76 | 34.6 ± 0.78 |
| **14** |  | 66.6 ± 0.92 | 82.2 ± 0.32 | 36.6 ± 0.11 |
| **15** |  | 33.2 ± 0.28 | 28.5 ± 0.21 | 17.8 ± 0.18 |
| **16** |  | 80.7 ± 0.83 | >100 | 90.0 ± 0.21 |
| **17** |  | 89.7 ± 0.02 | >100 | 54.3 ± 0.28 |
| **18** |  | 78.9 ± 0.73 | 98.0 ± 0.73 | >100 |
| **19** |  | 37.8 ± 0.82 | 40.5 ± 0.55 | 28.9 ± 0.94 |
| **20** |  | 56.4 ± 0.71 | 67.8 ± 0.24 | 31.2 ± 0.78 |
| **BHA (Butylated hydroxyanisole)c** | | 44.2 ± 0.24 |  |  |
| **Baicaleind** | | | 22.6 ± 0.08 |  |
| **Allopurinole** | | | | 2.9 ± 0.24 |

SEMa (Standard error of the mean); NAb (Not Active); BHA (Butylated hydroxyanisole)c Standard for antioxidant activity), Baicaleind (Standard for Lipoxygenase Inhibition Activity ) and Allopurinole (Standard for Xanthine Oxidase Inhibition Activity



**Figure-S1:** SAR of compounds **1-5**



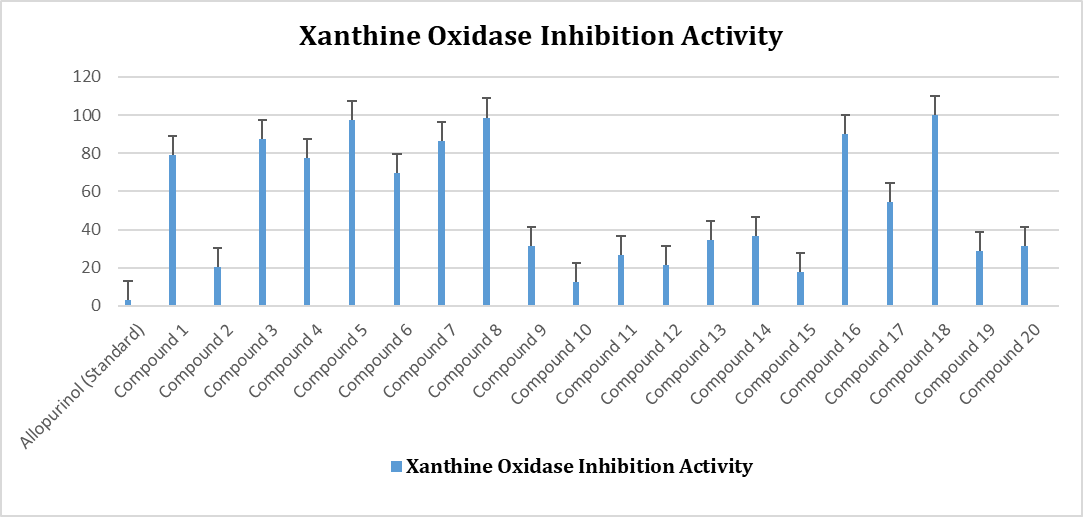
**Figure-S2:** SAR of compounds **6-11**



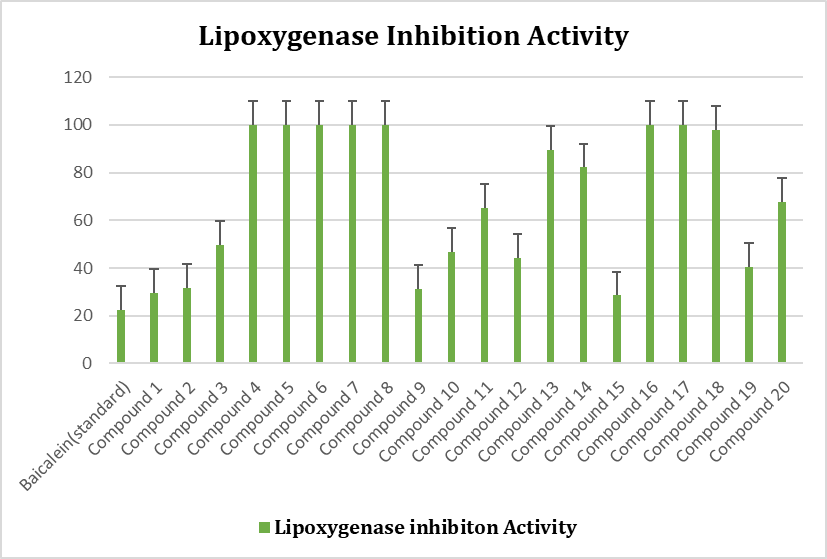
**Figure-S3:** SAR of compounds **12-15**



**Figure-S4:** SAR of compounds **16-20**

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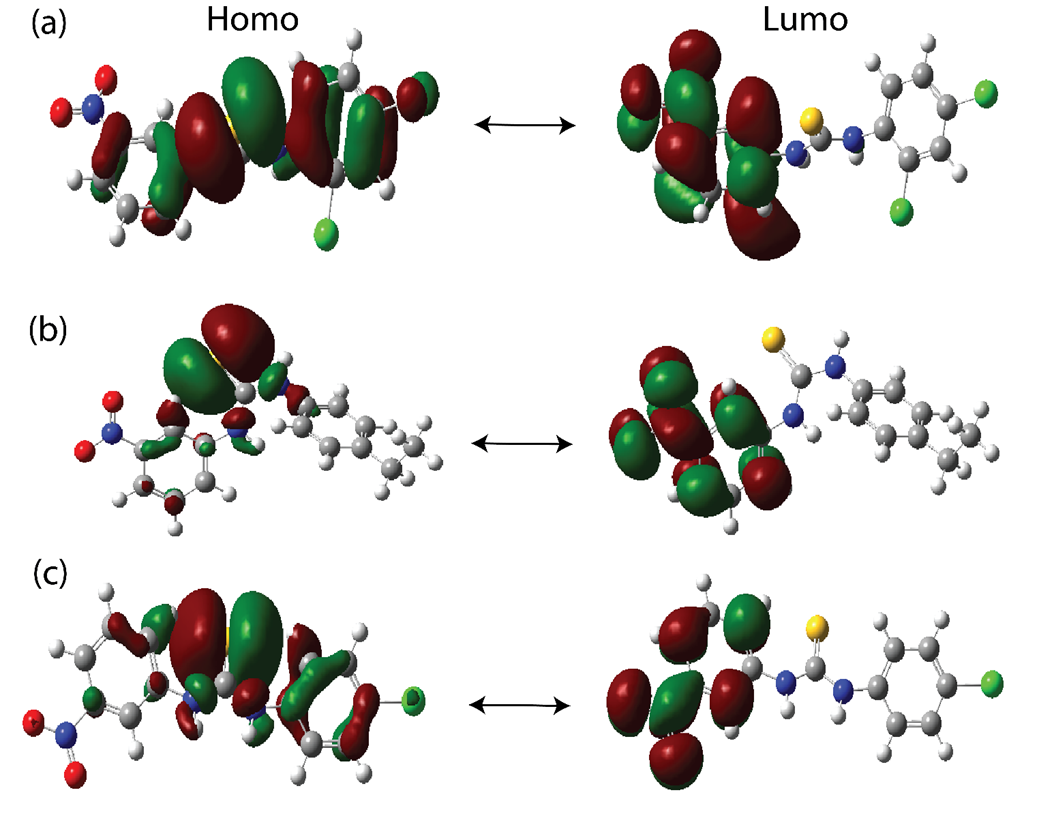
**Figure-S5:** Comparison of xanthine oxidase inhibition activity of compounds **1-20**

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**Figure-S6:** Comparison of lipoxygenase inhibition activity of compounds **1-20**

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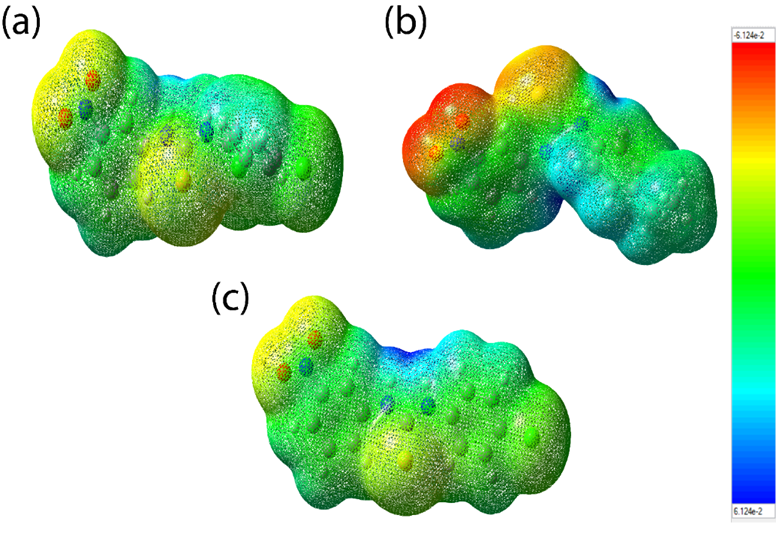
**Figure-S7**: Optimized structures at B3LYP/6-31G (d,p) level of theory (a) compound **1** (b) compound **2** (c) compound **3**



**Figure-S8**: HOMO-LUMO surfaces of compounds **1**, **2,** and **3**

**Table-S2:** Represent the Frontier orbitals energy, band gap, and global reactivity descriptors.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Comp. No** | **Homo**  **(eV)** | **Lumo**  **(eV)** | **Homo Lumo Energy Gap** | **Electron affinity (A)** | **Ionization potential (I)** | **Electronegativity (χ)** | **Chemical hardness (η)** |
| **1** | -8.9607 | -0.9992 | 7.9615 | 0.99 | 8.96 | 4.975 | 3.985 |
| **2** | -5.8205 | -2.7456 | 3.0749 | 2.74 | 5.82 | 4.28 | 1.54 |
| **3** | -6.111 | -3.0667 | 3.0449 | 3.06 | 6.11 | 4.585 | 1.52 |



**Figure-S9**: Represents the MESP surfaces for all compounds at the DFT level using the B3LYP/6-31G (d,p) method. Different colors indicate different electronegative regions; red is the most nucleophilic, dark blue is the most electrophilic region, and yellow, green, and light blue are in between

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**Figure-S10A-D**: The Docked conformations of most active thiourea derivatives against Lipoxygenase and Xanthine Oxidase. (**A**) the 3D binding mode of compound **1** in the active site Lipoxygenase, (**B**) the 3D binding mode of compound **15** in the active site Lipoxygenase, (**C**) the 3D binding mode of compound **10** in the active site Xanthine Oxidase, and (**D**) the 3D binding mode of compound **15** in the active site Xanthine Oxidase

**Table-S3:** Docking score and interaction details of compounds **1-20** with lipoxygenase enzyme.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compounds** | **Interacting residues** | **Interaction type** | **Distance** | **Energy** | **Docking Score** |
| **1** | Gln514  Gln514  Ala561 | H-donor  H-acceptor  H-acceptor | 3.13  3.58  3.25 | -1.4  -1.2  -0.5 | -6.89 |
| **2** | Gln514  His518 | H-donor  π-π | 2.89  3.92 | -0.5  -0.0 | -5.46 |
| **3** | Gln514  Ala561 | H-acceptor  H-acceptor | 3.57  3.29 | -1.2  -0.5 | -5.38 |
| **4** | Gln514  Ala561 | H-acceptor  H-acceptor | 3.54  3.64 | -1.2  -0.5 | -5.52 |
| **5** | Asp766  Gln514 | H-donor  H-acceptor | 3.58  3.56 | -0.5  -1.3 | -5.23 |
| **6** | Gln716  Thr575 | H-donor  H-donor | 3.18  3.36 | -2.6  -0.8 | -5.44 |
| **7** | His523 | H-π | 4.23 | -0.5 | -5.1 |
| **8** | Gln514 | H-acceptor | 3.84 | -1.0 | -4.98 |
| **9** | Gln716  Gln514 | H-donor  H-acceptor | 3.14  3.48 | -2.8  -1.2 | -5.32 |
| **10** | His518  His513 | H-donor  H-donor | 3.09  3.69 | -0.8  -1.2 | -5.73 |
| **11** | Gln716 | H-donor | 3.45 | -0.6 | -5.81 |
| **12** | His518  Trp519 | H-donor  H-acceptor | 3.26  3.40 | -1.0  -0.6 | -5.63 |
| **13** | Leu565 | π-H | 3.96 | -0.8 | -5.17 |
| **14** | Asn558 | H-donor | 3.55 | -0.9 | -4.97 |
| **15** | His518  His513  Ala561 | H-donor  H-acceptor  H-acceptor | 2.74  3.58  3.29 | -0.2  -0.4  -0.2 | -7.24 |
| **16** | His523 | H-π | 3.47 | -0.5 | -5.21 |
| **17** | His518 | H-donor | 2.87 | -1.5 | -5.28 |
| **18** | His513  Gln514 | H-donor  H-acceptor | 4.06  3.72 | -0.5  -0.6 | -5.49 |
| **19** | Ile572 | H-acceptor | 4.43 | -0.1 | -4.76 |
| **20** | Leu565  Ileπ572 | π-H  π-H | 3.40  3.74 | -0.2  -0.7 | -5.95 |

**Table-S4:** Docking score and interaction details of compounds **1-20** with xanthine oxidase enzyme.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compounds** | **Interacting residues** | **Interaction type** | **Distance** | **Energy** | **Docking Score** |
| **1** | Leu257  Val258 | H-donor  H-acceptor | 3.21  3.86 | -1.0  -0.5 | -5.62 |
| **2** | Lys256  Leu404  Leu404 | H-acceptor  H-acceptor  H-acceptor | 3.71  2.88  3.31 | -1.1  -2.0  -1.2 | -6.53 |
| **3** | Ala346  Gly350 | H-acceptor  π-H | 4.01  3.49 | -1.8  -0.6 | -5.52 |
| **4** | Gly350  Ile353 | H-acceptor  H-acceptor | 3.60  3.57 | -1.0  -0.7 | -5.36 |
| **5** | Asn261 | π-H | 3.60 | -0.6 | -4.95 |
| **6** | Thr354  Asn261 | H-donor  H-acceptor | 2.97  3.60 | -1.3  -0.5 | -5.44 |
| **7** | Leu257  Ile353 | π-H  π-H | 3.61  3.75 | -0.5  -0.6 | -5.43 |
| **8** | Thr354  Gly260 | H-donor  H-acceptor | 3.46  3.73 | -0.6  -0.7 | -5.68 |
| **9** | Leu257  Thr354 | H-donor  H-acceptor | 3.21  3.98 | -1.6  -0.5 | -5.62 |
| **10** | Leu257  Ala346  Asn351 | H-donor  H-acceptor  H-acceptor | 3.23  3.64  3.55 | -1.7  -0.5  -0.8 | -8.14 |
| **11** | Ile264  Val259 | H-acceptor  H-acceptor | 3.40  3.72 | -1.3  -1.4 | -5.81 |
| **12** | Leu404  Leu257 | H-acceptor  π-H | 3.47  3.87 | -0.5  -0.7 | -5.68 |
| **13** | Ser347  Val259 | H-acceptor  π-H | 3.51  3.90 | -0.5  -1.0 | -5.63 |
| **14** | Thr354  Val259 | H-donor  H-acceptor | 3.31  3.10 | -0.5  -1.2 | -5.76 |
| **15** | Ala346  Asn351  Asn261 | H-acceptor  H-acceptor  π-H | 3.62  3.59  3.87 | -0.5  -0.7  -0.5 | -7.57 |
| **16** | Val258  Asn261 | H-acceptor  H-acceptor | 3.90  3.21 | -0.9  -3.1 | -5.39 |
| **17** | Gly350  Asn261 | H-donor  H-acceptor | 3.39  3.42 | -0.8  -3.0 | -5.28 |
| **18** | Thr354  Ile264 | H-donor  H-acceptor | 3.10  3.55 | -0.7  -2.2 | -5.57 |
| **19** | Thr354 | H-acceptor | 4.02 | **-0.6** | -4.32 |
| **20** | Asn261 | π-H | 3.59 | -0.6 | -4.69 |