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Design, Synthesis, Characterization, Urease Inhibitory and Tyrosinase Inhibitory Activities of Some Chiral Pyrazolo Derivatives

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A series of some pyrazolo derivatives **(1-2)(a-f)** were synthesized and their urease inhibitory and tyrosinase inhibitory activities were evaluated. Among synthesized compounds, **2f**, **2e**, **1f**, **2d**, **1e**, **2b**, and **1d** showed excellent activities (IC_{50}) i.e. 11.73±0.28, 14.21±0.56, 15.37±0.89, 17.66±0.82, 18.64±0.19, 19.55±0.39, and 22.08±0.96 µM, respectively. According to tyrosinase assay results, compound **2f** showed the good tyrosinase inhibitory activity i.e. 6.12±0.40 mM of IC_{50}.

SYNTHESIS, CHARACTERIZATION, UREASE INHIBITORY AND TYROSINASE INHIBITORY ACTIVITIES OF SOME CHIRAL PYRAZOLO DERIVATIVES

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ABSTRACT

A series of some pyrazolo derivatives **(1-2)(a-f)** were synthesized and their urease inhibitory and tyrosinase inhibitory activities were evaluated. Among synthesized compounds, **2f**, **2e**, **1f**, **2d**, **1e**, **2b**, and **1d** showed excellent activities (IC_{50}) i.e. 11.73±0.28, 14.21±0.56, 15.37±0.89, 17.66±0.82, 18.64±0.19, 19.55±0.39, and 22.08±0.96 µM, respectively. According to tyrosinase assay results, compound **2f** showed the good tyrosinase inhibitory activity i.e. 6.12±0.40 mM of IC_{50}.

Keywords: Pyrrolidine, chiral, thiazolidine-2,4-one, 2-thioxothiazolidin-4-one, pyrazolo, urease inhibitory activity, tyrosinase inhibitory activity

INTRODUCTION

Pyrrolidines and derivatives, which it can be used for pharmaceutical purposes, are among the most bioactive *N*-heterocyclic compounds in organic chemistry as [1]. It is well known for their versatile pharmacological activities such as antimicrobial [2], antitumor [3], anticonvulsant [4], and anti-HIV [5]. The structures using barnidipine, tosufloxacin, alizapride, remoxipride,

triprolidine, profadol, and lanperisone of some drugs pyrrolidinyl containing are given in Figure 1.

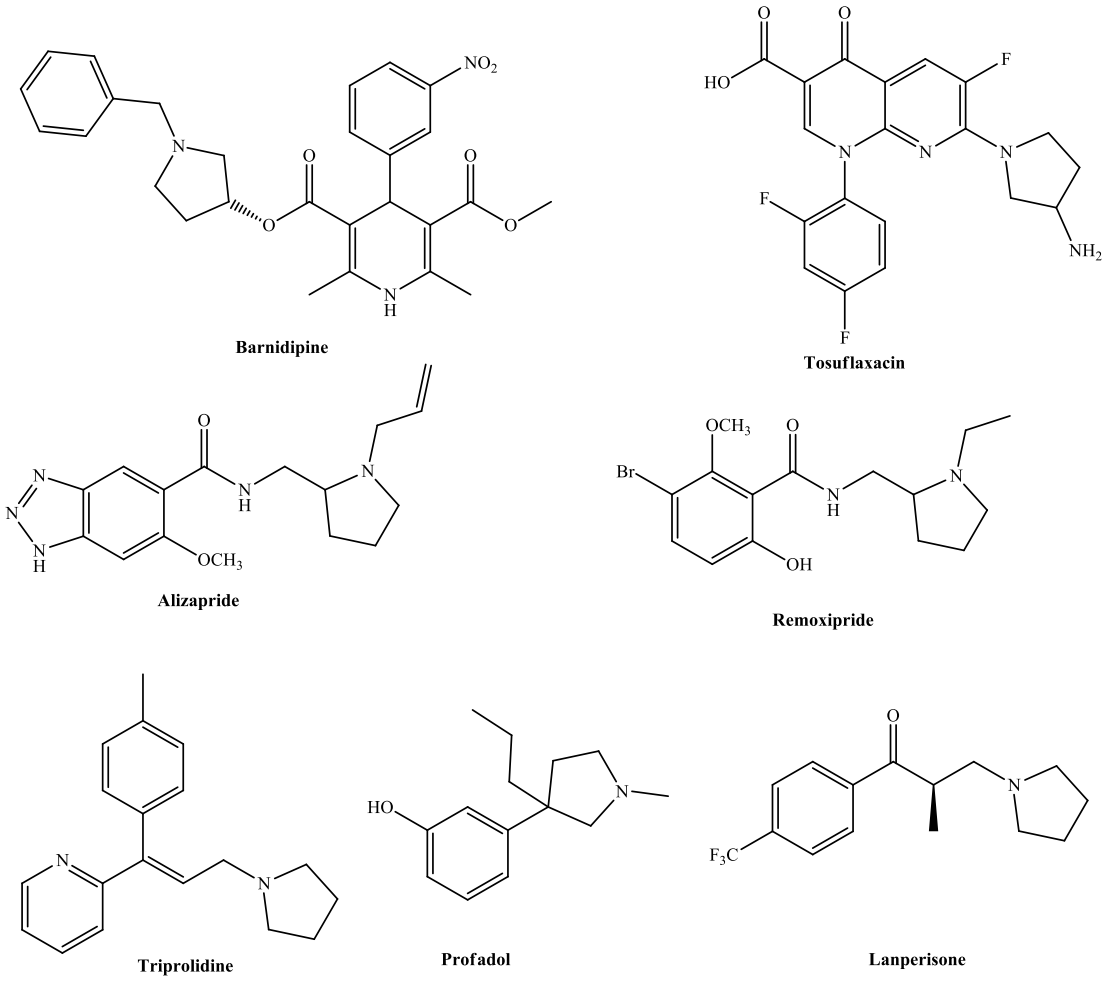

Figure 1. Pyrrolidinyl drugs

Thiazolidine and its derivatives are important drug candidates for anticancer [6], anti-HIV [7], anti-convulsant [8], sedative [9], antidepressant [10], antioxidant [11] activities and are a few among many other biologically important properties has by these promising compounds.

Pyrazole, which it clinically applicable, consist of one double band-unsaturated and 5-membered ring containing two nitrogen atoms. Pyrazole derivatives has been focused great attention as potent anti-inflammatory, analgesic and antipyretic agents [12, 13, 14]. The first pyrazole derivative using in treatment was antipyrine used in the treatment of pain, inflammation and

fever in 1884. The structures using celecoxib, rimonabant, antipyrine and ramifenazone some drugs pyrazole containing are given in Figure 2.

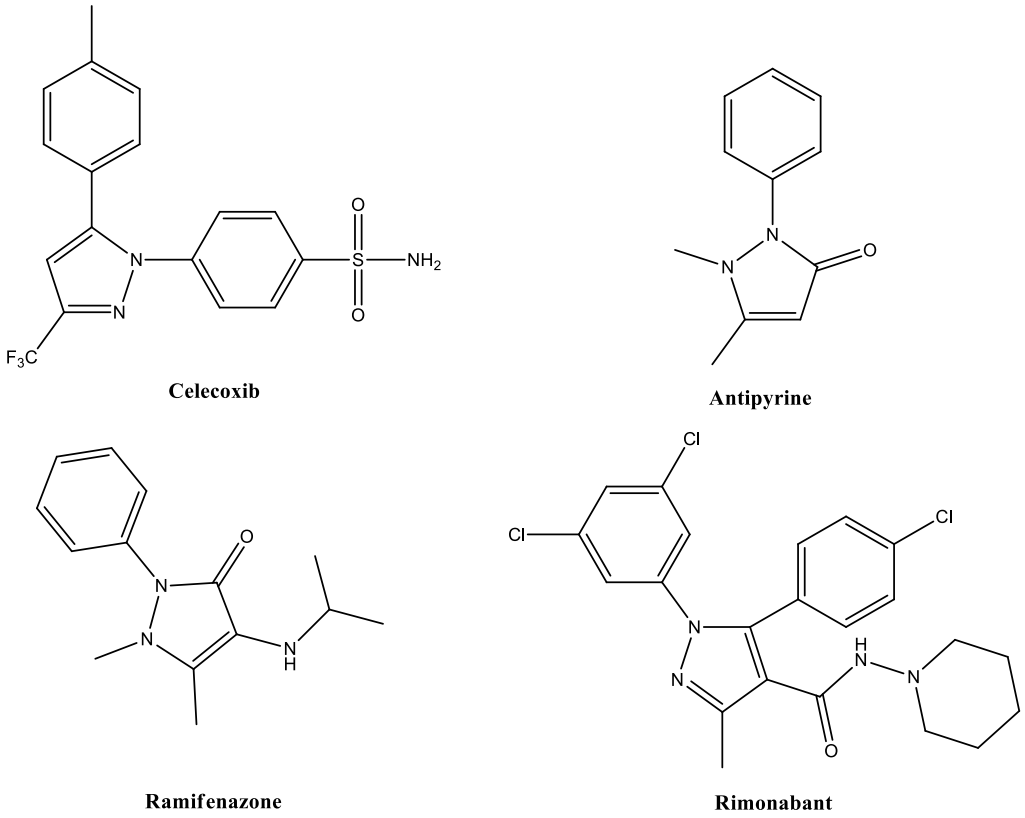

Figure 2. Pyrazole drugs

Urease (E.C. 3.5.1.5), isolated from bean seeds for the first time, is a metalloenzymide, also known as urea amidohydrolase, which contains nickel [15]. Urease enzyme hydrolyzes to urea as substrate and in result of hydrolization forms ammonia and carbon dioxide. Helicobacter pylori colonizes and emits urea enzyme produced by the production of CO_2 and NH_3 bacterium stomach fluid in the low pH to protect. However, NH_3 is toxic to stomach epithelial cells as well as enhances the effect of cytotoxins secreted by the agent by reducing intercellular adhesion. This can cause gastritis, peptic ulcer and gastric cancer. In this context, proton donor inhibitors and urease inhibitors are used in the treatment of *Helicobacter pylori* infection [16]. *N*-heterocyclic based agents used as urease inhibitors are given in Figure 3.

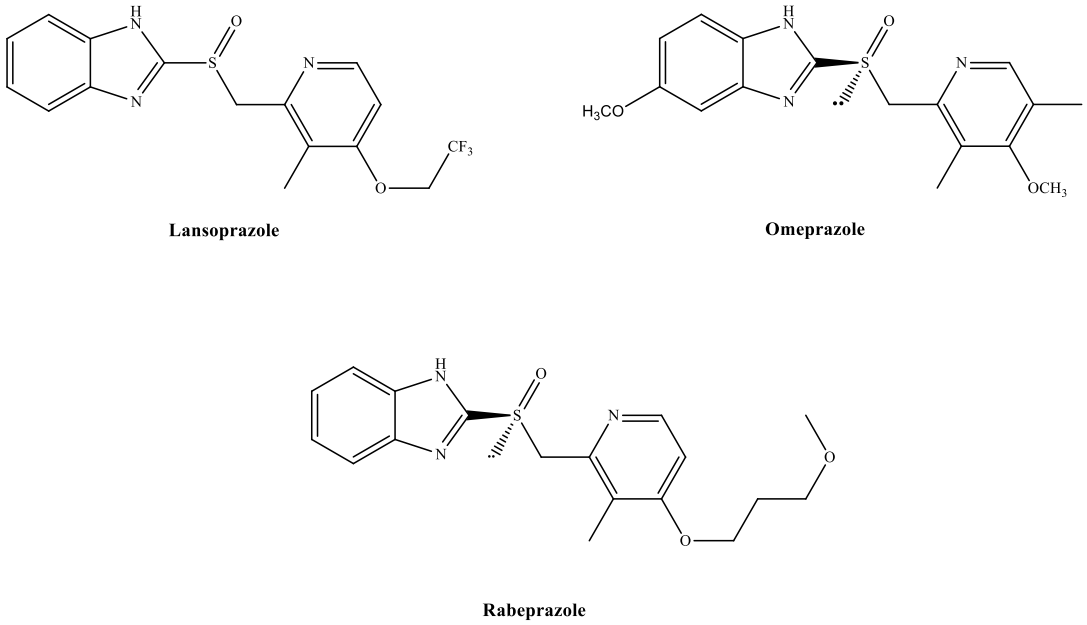

Figure 3. *N*-heterocyclic based urease **inhibitors**

Melanin, is one of the factors affecting on skin and hair color in humans, is produce a dark pigment to the innermost layers of the human epidermis. It's necessary to protect human skin against radiation. Upon exposure to UV radiation of the skin, melanogenesis is initiated through a tyrosinase enzyme [17]. Tyrosinase (EC 1.14.18.1) is a multifunctional and copper-containing enzyme [18]. Tyrosinase enzyme specifically rises the concentration of melanine pigment affect to tyrosine substrate [19]. Abnormal melanin pigmentation is a serious health problem on the skin in humans [20]. Tyrosinase inhibitors used for this purpose have clinical applications for the treatment of skin disorders related to melanin pigmentation. *N*-heterocyclic based agents used as tyrosinase inhibitors are given in Figure 4. Thus, new tyrosinase inhibitory agents has been avidly explored as an avenue for therapies to these diseases.

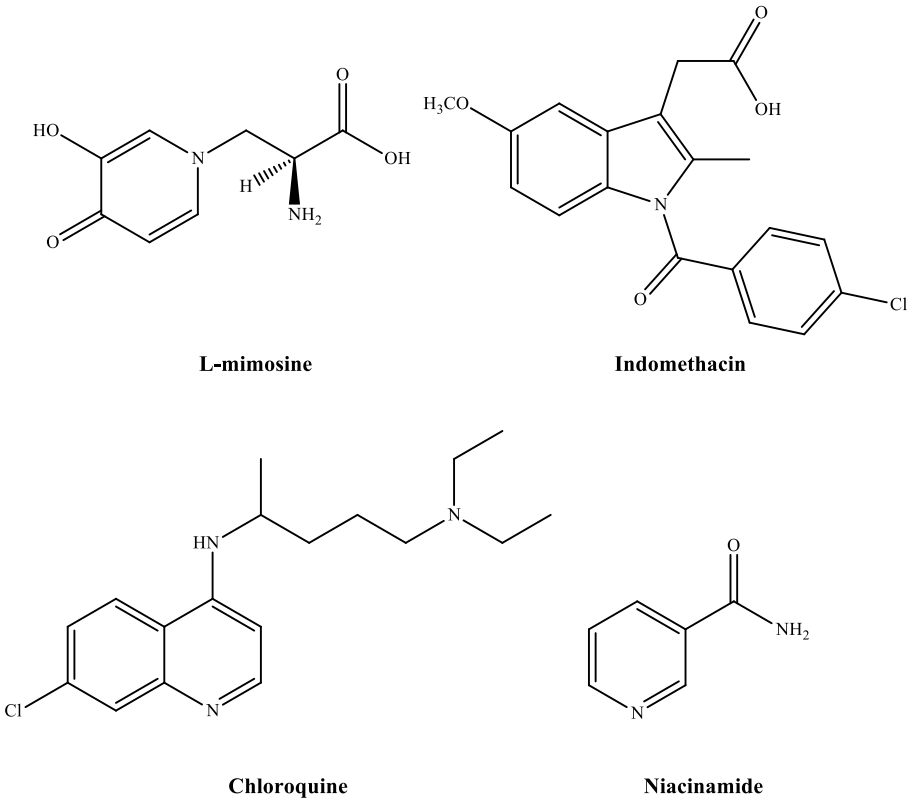

Figure 4. *N*-heterocyclic based tyrosinase inhibitors

In addition, the medicinal chemistry forms also about more than half of drug currently used in the treatment of chiral molecules in the discovery and development of new agents for treating diseases [21]. In view of these observations, design of new drug-like small molecules based on the pharmacologically attractive scaffolds of the pyrrolidine, thiazolidine and pyrazole in *N*-heterocyclic structure has always been a reasonable and promising direction in modern medicinal chemistry [22, 23]. The design strategy of target molecules was designed as the synthesis of new pyrazolo derivatives **(1-2)(a-f)** having a chiral carbons for new urease inhibitory and tyrosinase inhibitory agents for the first time.

RESULT & DISCUSSION

The *in vitro* urease inhibitory and tyrosinase inhibitory activity of pyrazolo-derived compounds **(1-2)(a-f)** reported with this study the first time. Synthetic route followed for the preparation of the target molecules are given in Figure 5.

Structures of pyrazolo derivatives **(1-2)(a-f)** were confirmed by analytical and spectral data. The C, H, N and S contents of prepared all compounds founded that it was consistent with their predicted structures. The contents of each compound C, H, S and N are given in the experimental part. The FTIR spectra founded band in region 1623-1663 cm^{-1} and 1539-1593 cm^{-1} corresponds to C=0 and C=N groups of thiazolidine and pyrazole in **(1-2)(a-f),** respectively. ^{1}H NMR spectra of the prepared compound **(1-2)(a-f)** showed dublet in range 4.16-4.44 and 3.92-4.38 ppm for C7 and C8 protons in pyrazolo ring. The ^{13}C NMR spectra of all the compound **(1-2)(a-f)** observed in range 50.8-64.4 and 54.8-60.3 ppm for C7 and C8 carbons in pyrazolo ring, respectively. All other signals are at their respective positions in ^{1}H NMR spectrum and ^{13}C NMR spectrum.

In general, 2-thioxothiazolidin-4-one-based pyrazolo derivatives **2(a-f)** exhibited better activity than thiazolidine-2,4-dione-based pyrazolo derivatives **1(a-f)**. The synthesized pyrazolo derivatives **(1-2)(a-f)** have been evaluated for their inhibitory effects on urease enzyme. All of the synthesized compounds demonstrated better urease inhibitory activity with IC_{50} value of ranging from 11.73±0.28 to 29.84±0.31 than thiourea with IC_{50} of 23.08±0.19 µM. Compound **2f**, **2e**, **1f**, **2d**, **1e**, **2b**, and **1d** were shown excellent activity IC_{50} value of 11.73±0.28, 14.21±0.56, 15.37±0.89, 17.66±0.82, 18.64±0.19, 19.55±0.39, and 22.08±0.96 µM, respectively. In this context, compound **2f**, **2e**, **1f**, **2d**, **1e**, **2b**, and **1d** could be converted into a usable product in pharmaceutical.

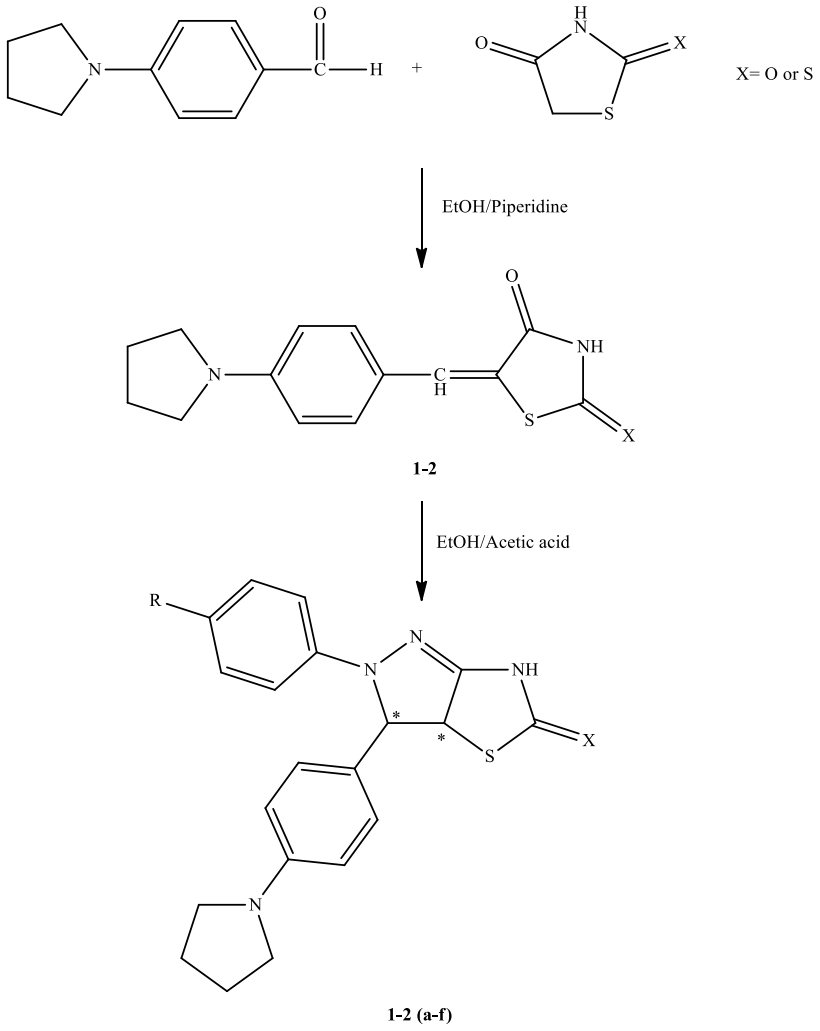

Figure 5. Synthetic route followed for the preparation of the target molecules

Tyrosinase inhibitors that used in the treatment of some dermatological disorders associated with melanin hyperpigmentation, have been an important role in the cosmetic and pharmaceutical industries for their skin-whitening effect sunburn [24]. The tyrosinase inhibitory activity results of pyrazolo derivatives **(1-2)(a-f)** given in Table 1. According to assay results, compound **2f**, **2e**, **1f,** and **2d** showed the best tyrosinase inhibitory activity IC_{50} value of 6.12±0.40, 8.45±0.13, 9.34±0.55, and 9.60±0.64 mM, respectively. In this context, it can be concluded that the synthesis of different pyrazolo derivatives due to the close proximity to the standards of the results can be a potential candidate for the treatment of melanin biosynthesis related skin disease.

Table 1. Urease inhibitory and tyrosinase inhibitory activities of synthesized pure compounds

Compound	*Urease Inhibitory Activity*	*Tyrosinase inhibitory activity*
	IC_{50} (µM)	*Tyrosinase assay IC_{50} (mM)*
1	30.27±0.46	22.06±0.17
2	29.98±0.44	20.72±0.41
1a	29.84±0.31	18.25±0.36
1b	24.73±0.93	15.79±0.43
1c	27.16±0.55	16.98±0.60
1d	22.08±0.96	12.37±0.28
1e	18.64±0.19	10.03±0.69
1f	15.37±0.89	9.34±0.55
2a	27.09±0.71	17.91±0.22
2b	19.55±0.39	13.15±0.07
2c	24.74±0.20	16.07±0.52
2d	17.66±0.82	9.60±0.64
2e	14.21±0.56	8.45±0.13
2f	11.73±0.28	6.12±0.40
***Thiourea*[b]**	23.08±0.19	NT
***Kojic acid*[b]**	NT	0.64±0.12
***L-mimosine*[b]**	NT	0.67±0.06

[a]Value represent the means ± standard deviation of three parallel measurements (p<0.05)

[b]Reference compound

NT: Not tested

EXPERIMENTAL

Chemicals and spectral measurements:

Ethanol (EtOH), piperidine, hydrazine monohydrate, acetic acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium hydroxide, sodium acetate, sodium hypochloride, urea, thiourea, phenol were obtained from E. Merck (Darmstadt, Germany). 4-iodophenylhydrazine, 4-(trifluoromethyl)phenylhydrazine, 4-nitrophenylhydrazine, Phenylhdyrazine, thiazolidine-2,4-dione, 4-(trifluoromethoxy)phenylhydrazine was obtained from Alfa Aesar Co., Inc.. 2-thioxothiazolidin-4-one, 3,4-dihydroxy-L-phenylalanine (L-DOPA), sodium nitroprusside dehydrate, 4-(1-pyrrolidinyl)benzaldehyde, urease [Type-III from Jack Beans, EC 232-656-0, 20990 U/g solid], and tyrosinase mushroom [EC 1.14.18.1, ≥1000 U/mg] were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany).

All of the reactions were monitored with Thin Layer Chromatography and the crude product purified by crystallization from appropriate solvents. The structures of synthesized compounds were elucidated by spectroscopy methods analyzed with FTIR, NMR techniques as ^{1}H NMR, ^{13}C

NMR. All the target pyrazolo derivatives **(1-2)(a-f)** were microanalyzed also satisfactorily for elemental analysis (C, H, N, S).

The spectra of synthesized compounds were recorded on Perkin Elmer 1620 model FT-IR spectrophotometer and Shimadzu IR-8400 spectrophotometer. Elemental analyses (C, H, N, S) were performed on a VarioMICRO elemental analyzer (Elementar Analysen Systeme, GmbH, Hanau, Germany). ^{1}H NMR spectra was obtained at room temperature with a Bruker Avance-DPX-400 NMR spectrometer (Bruker BioSpin, Billerca, USA) in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. ^{13}C NMR were spectra recorded on a Agilent Techonologies with 150 MHz NMR. Bioactivity assay measurements were carried out on a 96-well microplate reader, SpectraMax 340PC^{384}, Molecular Devices (USA), at Department of Chemistry, Muğla Sıtkı Koçman University.

Synthesis

General synthesis procedure of compound (1-2)

A mixture of thiazolidine-2,4-dione or 2-thioxothiazolidin-4-one (20 mmol) 4-(1-pyrrolidinyl)benzaldehyde (20 mmol), piperidine (16 mmol) and EtOH (50 mL) was refluxed for 24 h. The reaction mixture was poured into H_2O and acidified with AcOH to give compound **1-2** as solids which were recrystallized from ethanol [25].

General synthesis procedure of pyrazolo derivatives (1-2)(a-f)

A mixture of 5 mmol 5-(4-(pyrrolidin-1-yl)benzylidene)thiazolidin-2,4-dione **(1)** or 5-(4-(pyrrolidin-1-yl)benzylidene)-2-thioxothiazolidin-4-one **(2)** with different hydrazines (5 mmol) were refluxed with sodium acetate (2 g) in absolute ethanol (20 mL). The product so obtained was, separated, washed and crystallised from glacial acetic acid [26].

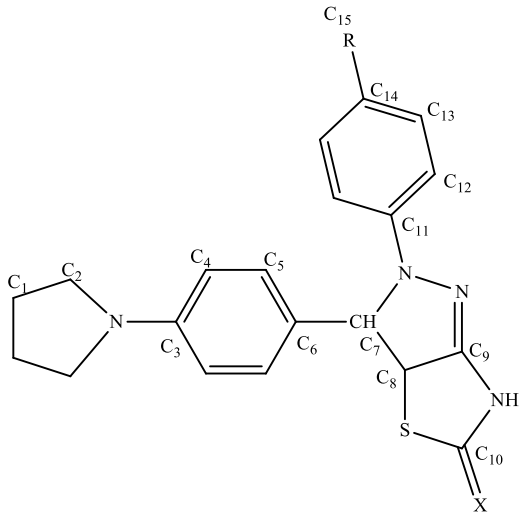

Figure 6. Locations of carbons used to illuminate target products

5-(4-(pyrrolidin-1-yl)benzylidene)thiazolidin-2,4-dione (1):

Yield: 74%; claret red solid, m.p. 141.0-141.7°C. IR ($\bar{\upsilon}$, cm^{-1}): 3279, 3228 (asym and sym N-H), 3063 (aromatic C-H), 2966, 2922 (aliphatic C-H), 1668-1633 (C=O), 1523 (C=N). ^{1}H-NMR (DMSO-*d*$_6$/TMS, 600 MHz): δ (ppm): 1.90 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.46 (t, *J*=4.8 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 6.74 (d, *J*$_1$=12.0 *Hz*, 2H, *o*-ArH of pyyrolidine-C**H**-), 7.74 (d, *J*$_1$=12.0 *Hz*, 2H, *m*-ArH of pyyrolidine-C**H**-), 7.77 (s, 1H, -C**H**-), 12.40 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-*d*$_6$/TMS, 150 MHz): δ (ppm): 25.7 (C$_1$), 50.9 (C$_2$), 111.8 (C$_4$), 117.4 (C$_8$), 124.8 (C$_6$), 130.1 (C$_5$), 143.8 (C$_7$), 148.9 (C$_3$), 167.4 (C$_9$), 167.9 (C$_{10}$). Anal. calcd. for $C_{14}H_{14}N_2O_2S$: C, 61.29; H, 5.14; N, 10.21; S, 11.69 %. Found: C, 61.17; H, 5.01; N, 10.12; S, 11.60 %.

5-(4-(pyrrolidin-1-yl)benzylidene)-2-thioxothiazolidin-4-one (2):

Yield: 70%; yellow solid, m.p. 148.8-149.2°C. IR ($\bar{\upsilon}$, cm^{-1}): 3270, 3215 (asym and sym N-H), 3040 (aromatic C-H), 2954, 2920 (aliphatic C-H), 1675 (C=O), 1529 (C=N). ^{1}H-NMR (DMSO-*d*$_6$/TMS, 600 MHz): δ (ppm): 1.89 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.45 (t, *J*=3.6 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 6.70 (d, *J*$_1$=12.0 *Hz*, 2H, *o*-ArH of pyyrolidine-C**H**-), 7.73 (d, *J*$_1$=12.0 *Hz*, 2H, *m*-ArH of pyyrolidine-C**H**-), 7.75 (s, 1H, -C**H**-), 12.41 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-*d*$_6$/TMS, 150 MHz): δ (ppm): 25.9 (C$_1$), 50.8 (C$_2$), 111.9 (C$_4$), 117.4 (C$_8$), 124.9 (C$_6$), 130.4 (C$_5$), 143.6 (C$_7$), 148.5 (C$_3$), 169.1 (C$_9$), 194.1 (C$_{10}$). Anal. calcd. for $C_{14}H_{14}N_2OS_2$: C, 57.90; H, 4.86; N, 9.65; S, 22.08 %. Found: C, 56.28; H, 4.87; N, 9.58; S, 22.00 %.

3-(4-(pyrrolidin-1-yl)phenyl)-3-3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(6*H*)-one (1a)

Yield: 20 %; red solid, m.p. 173.1°C. IR ($\bar{\upsilon}$, cm^{-1}): 3240, 3210 (asym and sym N-H); 3072 (aromatic C-H); 2951, 2936 (asym and sym of aliphatic C-H); 1654 (C=O); 1593 (C=N). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz): δ (ppm): 1.90 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.55 (t, *J*=4.0 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 4.16 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 4.20 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 6.75 (d, 2H, J_1=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 7.14 (d, J_1=12.0 *Hz*, 2H, *m*-ArH of pyyrolidine-C**H**-), 11.80 (d, 1H, J_1=10.0 *Hz*, -CH-N**H**-), 12.65 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz): δ (ppm): 28.0 (C$_1$), 50.8 (C$_7$), 51.4 (C$_2$), 56.5 (C$_8$), 113.1 (C$_4$), 130.2 (C$_5$), 134.1 (C$_6$), 151.8 (C$_3$), 158.9 (C$_9$), 178.0 (C$_{10}$). Anal. calcd. for $C_{14}H_{16}N_4OS$: C, 58.31; H, 5.59; N, 19.43; S, 11.12%. Found: C, C, 58.37; H, 5.66; N, 19.52; S, 11.19 %.

2-phenyl-3-(4-(pyrrolidin-1-yl)phenyl)-3,3a-dihydro-*2H*-pyrazolo[3,4-*d*]thiazol-5(*6H*)-one (1b)

Yield: 28 %; red solid, m.p. 175.0°C. IR ($\bar{\upsilon}$, cm^{-1}): 3232, 3202 (asym and sym N-H); 3061 (aromatic C-H); 2957, 2912 (asym and sym of aliphatic C-H); 1629 (C=O); 1539 (C=N). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz): δ (ppm): 1.94 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.48 (t, *J*=3.6 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 4.29 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 4.38 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 6.77 (d, 2H, J_1=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 6.80 (dd, 1H, J_1=7.2 *Hz*, J_2=7.2 *Hz*, *p*-ArH of *N*-phenyl-C**H**-), 6.91 (d, 2H, *J*= 8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 7.16 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.28 (dd, 2H, J_1= 7.2 *Hz*, J_2=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.65 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz): δ (ppm): 27.2 (C$_1$), 51.2 (C$_2$), 54.8 (C$_8$), 61.4 (C$_7$), 114.8 (C$_4$), 118.7 (C$_{12}$), 131.2 (C$_5$), 133.5 (C$_{13}$), 134.4 (C$_6$), 148.6 (C$_{11}$), 153.8 (C$_3$), 160.1 (C$_9$), 177.9 (C$_{10}$). Anal. calcd. for $C_{20}H_{20}N_4OS$: C, 65.91; H, 5.53; N, 15.37; S, 8.80 %. Found: C, 66.87; H, 5.60; N, 15.48; S, 9.01 %.

2-(4-iodophenyl)-3-(4-(pyrrolidin-1-yl)phenyl)-3,3a-dihydro-*2H*-pyrazolo[3,4-*d*]thiazol-5(*6H*)-one (1c)

Yield: 22 %; red solid, m.p. 151.8°C. IR ($\bar{\upsilon}$, cm^{-1}): 3232, 3202 (asym and sym N-H); 3061 (aromatic C-H); 2957, 2912 (asym and sym of aliphatic C-H); 1623 (C=O); 1540 (C=N). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz): δ (ppm): 1.96 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.51 (t, *J*=4.2 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 3.99 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 4.36 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 6.70 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 6.99 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-),

7.22 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.36 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.78 (s, 1H, -N**H**-).^{13}C-NMR (DMSO-d_6/TMS, 150 MHz): δ (ppm): 26.8 (C_1), 51.8 (C_2), 59.5 (C_8), 63.3 (C_7), 78.9 (C_{14}), 116.2 (C_4), 119.1 (C_{12}), 132.6 (C_5), 136.1 (C_{13}), 139.7 (C_6), 147.0 (C_{11}), 155.2 (C_3), 162.3 (C_9), 205.1 (C_{10}). Anal. calcd. for $C_{20}H_{19}IN_4OS$: C, 48.99; H, 3.91; N, 11.43; S, 6.54 %. Found: C, 49.87; H, 3.88; N, 11.55; S, 6.66 %.

3-(4-(pyrrolidin-1-yl)phenyl)-2-(4-(trifluoromethyl)phenyl)-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(6*H*)-one (1d)

Yield: 24 %; red solid, m.p. 161.4°C. IR ($\bar{\upsilon}$, cm^{-1}): 3241, 3230 (asym and sym N-H); 3090 (aromatic C-H); 2960, 2939 (asym and sym o faliphatic C-H); 1663 (C=O); 1560 (C=N). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz): δ (ppm): 2.01 (m, 4H, -C**H**$_2$-CH_2-N-), 3.52 (t, *J*=4.0 Hz, 4H, -CH_2-C**H**$_2$-N-), 4.16 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 4.44 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 6.55 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 6.74 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 7.18 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.52 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.80 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz): δ (ppm): 26.5 (C_1), 53.6 (C_2), 56.4 (C_8), 63.5 (C_7), 115.1 (C_4), 118.3 (C_{12}), 124.0 (C_{15}), 126.2 (C_{14}), 128.0 (C_{13}), 130.4 (C_5), 136.2 (C_6), 149.1 (C_{11}), 152.9 (C_3), 164.8 (C_9), 181.7 (C_{10}). Anal. calcd. for $C_{21}H_{19}F_3N_4OS$: C, 58.32; H, 4.43; N, 12.96; S, 7.41 %. Found: C, 58.88; H, 4.45; N, 12.73; S, 7.32 %.

3-(4-(pyrrolidin-1-yl)phenyl)-2-(4-(trifluoromethoxy)phenyl)-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(6*H*)-one (1e)

Yield: 33 %; red solid, m.p. 155.9°C. IR ($\bar{\upsilon}$, cm^{-1}): 3235, 3224 (asym and sym N-H); 3084 (aromatic C-H); 2954, 2933 (asym and sym aliphatic C-H); 1657 (C=O); 1554 (C=N). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz): δ (ppm): 1.92 (m, 4H, -C**H**$_2$-CH_2-N-), 3.56 (t, *J*=3.8 Hz, 4H, -CH_2-C**H**$_2$-N-), 4.08 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 4.25 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 6.54 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 6.92 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 6.90 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 7.29 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 12.68 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz): δ (ppm): 26.6 (C_1), 52.4 (C_2), 55.2 (C_8), 63.8 (C_7), 116.8 (C_4), 117.5 (C_{12}), 128.4 (C_{13}), 132.3 (C_5), 134.6 (C_{15}), 136.8 (C_6), 139.0 (C_{11}), 142.7 (C_{14}), 154.6 (C_3), 166.2 (C_9), 178.5 (C_{10}). Anal. calcd. for $C_{21}H_{19}F_3N_4O_2S$: C, 56.24; H, 4.27; N, 12.49; S, 7.15 %. Found: C, 57.03; H, 4.33; N, 12.62; S, 7.29 %.

2-(4-nitrophenyl)-3-(4-(pyrrolidin-1-yl)phenyl)-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(6*H*)-one (1f)

Yield: 38 %; bordo solid, m.p. 173.8°C. IR ($\bar{\upsilon}$, cm^{-1}): 3232, 3221 (asym and sym N-H); 3081 (aromatic C-H); 2951, 2930 (asym and sym of aliphatic C-H); 1654 (C=O); 1551 (C=N). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz): δ (ppm): 2.00 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.59 (t, *J*=4.0 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 4.20 (d, 1H, *J*=.6.0 *Hz*, -CH-C**H**-S-), 4.36 (d, 1H, *J*=5.6 *Hz*, -C**H**-CH-S-), 6.80 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 7.22 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.30 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 8.11 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.80 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz): δ (ppm): 27.8 (C_1), 54.6 (C_2), 58.6 (C_8), 63.1 (C_7), 118.1 (C_4), 119.9 (C_{12}), 126.8 (C_{13}), 132.4 (C_5), 137.6 (C_6), 140.1 (C_{14}), 142.8 (C_{11}), 156.1 (C_3), 166.0 (C_9), 180.0 (C_{10}). Anal. calcd. for $C_{20}H_{19}N_5O_3S$: C, 58.67; H, 4.68; N, 17.10; S, 7.83 %. Found: C, 58.91; H, 4.66; N, 17.17; S, 7.88 %.

3-(4-(pyrrolidin-1-yl)phenyl)-3-3a-dihydro-*2H*-pyrazolo[3,4-*d*]thiazol-5(*6H*)-thione (2a)

Yield: 32 %; yellow solid, m.p. 178.3°C. IR ($\bar{\upsilon}$, cm-1): 3273, 3218 (asym and sym N-H); 3043 (aromatic C-H); 2957, 2923 (asym and sym of aliphatic C-H); 1554 (C=N). ^{1}H-NMR (DMSO-d6/TMS, 600 MHz): δ (ppm): 1.95 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.59 (t, *J*=4.2 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 3.92 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 4.33 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 6.74 (d, 2H, J_1=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 7.13 (d, J_1=12.0 *Hz*, 2H, *m*-ArH of pyyrolidine-C**H**-), 11.68 (d, 1H, J_1=10.0 *Hz*, -CH-N**H**-), 12.77 (s, 1H, -N**H**-).^{13}C-NMR (DMSO-d6/TMS, 150 MHz): δ (ppm): 26.2 (C_1), 52.6 (C_7), 54.6 (C_2), 60.3 (C_8), 112.9 (C_4), 130.7 (C_5), 134.8 (C_6), 151.6 (C_3), 159.4 (C_9), 201.2 (C_{10}). Anal. calcd. for $C_{14}H_{16}N_4S_2$: C, 55.23; H, 5.30; N, 18.40; S, 21.07 %. Found: C, 55.33; H, 5.36; N, 18.52; S, 21.15%.

2-phenyl-3-(4-(pyrrolidin-1-yl)phenyl)-3,3a-dihydro-*2H*-pyrazolo[3,4-*d*]thiazol-5(*6H*)-thione (2b)

Yield: 41 %; yellow solid, m.p. 181.1°C. IR ($\bar{\upsilon}$, cm^{-1}): 3265, 3210 (asym and sym N-H); 3035 (aromatic C-H); 2959, 2915 (asym and sym of aliphatic C-H); 1546 (C=N). ^{1}H-NMR (DMSO-d_6/TMS, 600 MHz): δ (ppm): 1.96 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.51 (t, *J*=3.6 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 3.99 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 4.36 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 6.70 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 6.84 (dd, 1H, J_1=7.2 *Hz*, J_2=7.2 *Hz*, *p*-ArH of *N*-phenyl-C**H**-),

6.99 (d, 2H, *J*= 8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 7.22 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.36 (dd, 2H, J_1= 7.2 *Hz*, J_2= 8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.78 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d_6/TMS, 150 MHz): δ (ppm): 26.8 (C_1), 51.8 (C_2), 59.5 (C_8), 63.3 (C_7), 116.2 (C_4), 119.1 (C_{12}), 132.6 (C_5), 136.1 (C_{13}), 139.7 (C_6), 147.0 (C_{11}), 155.2 (C_3), 162.3 (C_9), 205.1 (C_{10}). Anal. calcd. for $C_{20}H_{20}N_4S_2$: C, 63.13; H, 5.30; N, 14.72; S, 16.85 %. Found: C, 63.27; H, 5.42; N, 14.66; S, 16.71%.

2-(4-iodophenyl)-3-(4-(pyrrolidin-1-yl)phenyl)-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(*6H*)-thione (2c)

Yield: 35 %; yellow solid, m.p. 179.2°C. IR (ῡ, cm-1): 3272, 3216 (asym and sym N-H); 3041 (aromatic C-H); 2966, 2921 (asym and sym of aliphatic C-H); 1553 (C=N). ^{1}H-NMR (DMSO-d6/TMS, 600 MHz): δ (ppm): 1.98 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.48 (t, *J*=4.6 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 3.94 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 4.33 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 6.72 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 7.09 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 7.25 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.48 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.80 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d6/TMS, 150 MHz): δ (ppm): 27.2 (C_1), 51.4 (C_2), 58.8 (C_8), 62.8 (C_7), 70.9 (C_{14}), 113.4 (C_4), 116.5 (C_{12}), 132.6 (C_5), 135.3 (C_6), 139.0 (C_{13}), 143.8 (C_{11}), 154.2 (C_3), 160.3 (C_9), 202.5 (C_{10}). Anal. calcd. for $C_{20}H_{19}IN_4S_2$: C, 47.43; H, 3.78; N, 11.06; S, 12.66 %. Found: C, 47.87; H, 3.65; N, 11.28; S, 12.57 %.

3-(4-(pyrrolidin-1-yl)phenyl)-2-(4-(trifluoromethyl)phenyl)-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(*6H*)-thione (2d)

Yield: 33%; yellow solid, m.p. 152.8°C. IR (ῡ, cm-1): 3276, 3220 (asym and sym N-H); 3045 (aromatic C-H); 2971, 2926 (asym and sym of aliphatic C-H); 1557 (C=N). ^{1}H-NMR (DMSO-d6/TMS, 600 MHz): δ (ppm): 1.99 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.60 (t, *J*=4.0 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 3.92 (d, 1H, *J*=8.0 *Hz*, -CH-C**H**-S-), 4.32 (d, 1H, *J*=8.0 *Hz*, -C**H**-CH-S-), 6.64 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 6.80 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 7.22 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.50 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.80 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d6/TMS, 150 MHz): δ (ppm): 27.2 (C_1), 53.1 (C_2), 58.8 (C_8), 64.3 (C_7), 116.4 (C_4), 118.0 (C_{12}), 126.2 (C_{15}), 126.9 (C_{14}), 129.2 (C_{13}), 132.3 (C_5), 137.4 (C_6), 151.0 (C_{11}), 153.4 (C_3), 162.1 (C_9), 200.7 (C_{10}). Anal. calcd. for $C_{21}H_{19}F_3N_4S_2$: C, 56.23; H, 4.27; N, 12.49; S, 14.30 %. Found: C, 56.56; H, 4.38; N, 12.57; S, 14.63 %.

3-(4-(pyrrolidin-1-yl)phenyl)-2-(4-(trifluoromethoxy)phenyl)-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(6*H*)-thione (2e)

Yield: 42 %; yellow solid, m.p. 155.5°C. IR (ῡ, cm-1): 3272, 3216 (asym and sym N-H); 3041 (aromatic C-H); 2966, 2921 (asym and sym of aliphatic C-H); 1553 (C=N). ^{1}H-NMR (DMSO-d6/TMS, 600 MHz): δ (ppm): 2.05 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.52 (t, *J*=4.2 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 4.12 (d, 1H, *J*=6.0 *Hz*, -CH-C**H**-S-), 4.30 (d, 1H, *J*=5.6 *Hz*, -C**H**-CH-S-), 6.66 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of *N*-phenyl-C**H**-), 6.82 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 6.94 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 7.38 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 12.78 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d6/TMS, 150 MHz): δ (ppm): 26.6 (C$_1$), 53.3 (C$_2$), 59.6 (C$_8$), 64.4 (C$_7$), 117.6 (C$_4$), 118.2 (C$_{12}$), 120.9 (C$_{13}$), 134.9 (C$_{15}$), 135.2 (C$_5$), 138.2 (C$_6$), 139.9 (C$_{11}$), 142.4 (C$_{14}$), 155.8 (C$_3$), 163.1 (C$_9$), 202.1 (C$_{10}$).Anal. calcd. for $C_{21}H_{19}F_3N_4OS_2$: C, 54.30; H, 4.12; N, 12.06; S, 13.81 %. Found: C, 53.88; H, 4.28; N, 12.11; S, 14.09 %.

2-(4-nitrophenyl)-3-(4-(pyrrolidin-1-yl)phenyl)-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*]thiazol-5(6*H*)-thione (2f)

Yield: 40 %; yellow solid, m.p. 163.1 °C. IR (ῡ, cm-1): 3277, 3218 (asym and sym N-H); 3034 (aromatic C-H); 2952, 2933 (asym and sym of aliphatic C-H); 1545 (C=N). ^{1}H-NMR (DMSO-d6/TMS, 600 MHz): δ (ppm): 2.02 (m, 4H, -C**H**$_2$-CH$_2$-N-), 3.60 (t, *J*=4.0 Hz, 4H, -CH$_2$-C**H**$_2$-N-), 4.18 (d, 1H, *J$_1$*=.6.0 *Hz*, -CH-C**H**-S-), 4.44 (d, 1H, *J*=5.6 *Hz*, -C**H**-CH-S-), 6.82 (d, 2H, *J*=12.0 *Hz*, *o*-ArH of pyyrolidine-C**H**-), 7.26 (d, 2H, *J*=12.0 *Hz*, *m*-ArH of pyyrolidine-C**H**-), 7.34 (d, 2H, *J*=8.0 *Hz*, *o*-ArH of N-phenyl-C**H**-), 8.12 (d, 2H, *J*=8.0 *Hz*, *m*-ArH of *N*-phenyl-C**H**-), 12.84 (s, 1H, -N**H**-). ^{13}C-NMR (DMSO-d6/TMS, 150 MHz): δ (ppm): 26.4 (C$_1$), 52.9 (C$_2$), 59.3 (C$_8$), 64.4 (C$_7$), 115.2 (C$_4$), 116.3 (C$_{12}$), 127.4 (C$_{13}$), 133.6 (C$_5$), 138.1 (C$_6$), 141.2 (C$_{14}$), 144.7 (C$_{11}$), 155.5 (C$_3$), 165.3 (C$_9$), 202.2 (C$_{10}$). Anal. calcd. for $C_{20}H_{19}N_5O_2S_2$: C, 56.45; H, 4.50; N, 16.46; S, 15.07 %. Found: C, 56.64; H, 4.65; N, 17.05; S, 15.08 %.

Biological Activities

Solutions of pyrazolo derivatives compound **(1-2)(a-f)** were prepared at four different concentrations as 400-200-100-50 µM for urease inhibitory assay and 400-200-100-50 mM for tyrosinase inhibitory assay in EtOH. EtOH was used as a control, while thiourea and kojic acid with L-mimosine were used as urease and tyrosinase standards for comparison of the activity

tests. The results were given as 50% concentration (IC_{50}) for urease inhibitory and tyrosinase inhibitory activities assay.

The spectrophotometric analysis of urease inhibitory and tyrosinase inhibitory activities were performed according to the literature procedures as follows: by measuring ammonia production using the indophenol method as described by [27] and Hearing method with slight modification by [24], respectively.

Statistical analysis

All data on biological activity assay studies were the averages of triplicate analyses. All biological activity assays were carried out at four concentrations, and the results are presented as 50% concentration (IC_{50}) (%). Data were recorded as mean ± SEM (standard error of the mean). Significant differences between means were determined by Student's-*t* test and *p* values <0.05 were regarded as significant.

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