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ORIGINAL RESEARCH

The synthesis and antitubercular activity of substituted hydrazone,2-pyrazoline-5-one and 2-isoxazoline-5-one derivatives possessing 1,3,4-thiadiazole moiety

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ABSTRACT: Novel derivatives of substituted hydrazone (2a-e), 2-pyrazoline-5-one (3a-e, 4a-e) and 2-isoxazoline-5-one (5a-e) derivatives possessing 1,3,4-thiadiazole moiety were synthesized and evaluated for their antitubercular activity. The highest inhibitions were observed with the synthesized compounds are 87% for 3-methyl-4-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl)phenylhydrazono]-2-isoxazoline-5-one (5b) and 86% for ethyl 2-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl)phenylhydrazono]-3-oxobutirate (2b). Compounds 2b and 5b could be a good initial point to develop new lead compound.

KEY WORDS: 1,3,4-thiadiazole, 2-pyrazolin-5-one, 2-isoxazoline-5-one, hydrazone and antitubercular activity

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INTRODUCTION

Tuberculosis continues to be one of the major health problems in the world. One-third of the world's population is infected with *Mycobacterium tuberculosis* (1,2). Therefore, the identification of new compounds for the treatment of tuberculosis is an important under taking in medicinal chemistry research. 1,3,4-Thiadiazole derivatives have received much attention due o their versatile biological activities as antibacterial (3-5), antitubercular (6-10), antifungal (11), antiviral (12,13), anticancer (3,14-17), antiproliferative (18), antiinflammatory, analgesic and antipyretic (19). Pyrazolinones have also shown various biological activities (20-22). Linezolid (Zyvox^{TM}) is the first marketed oxazolidinone derivative for antibacterial (23) and antitubercular (24,25) infections. In the view of these above mentioned facts and attempt to achieve new compounds with better antitubercular properties.

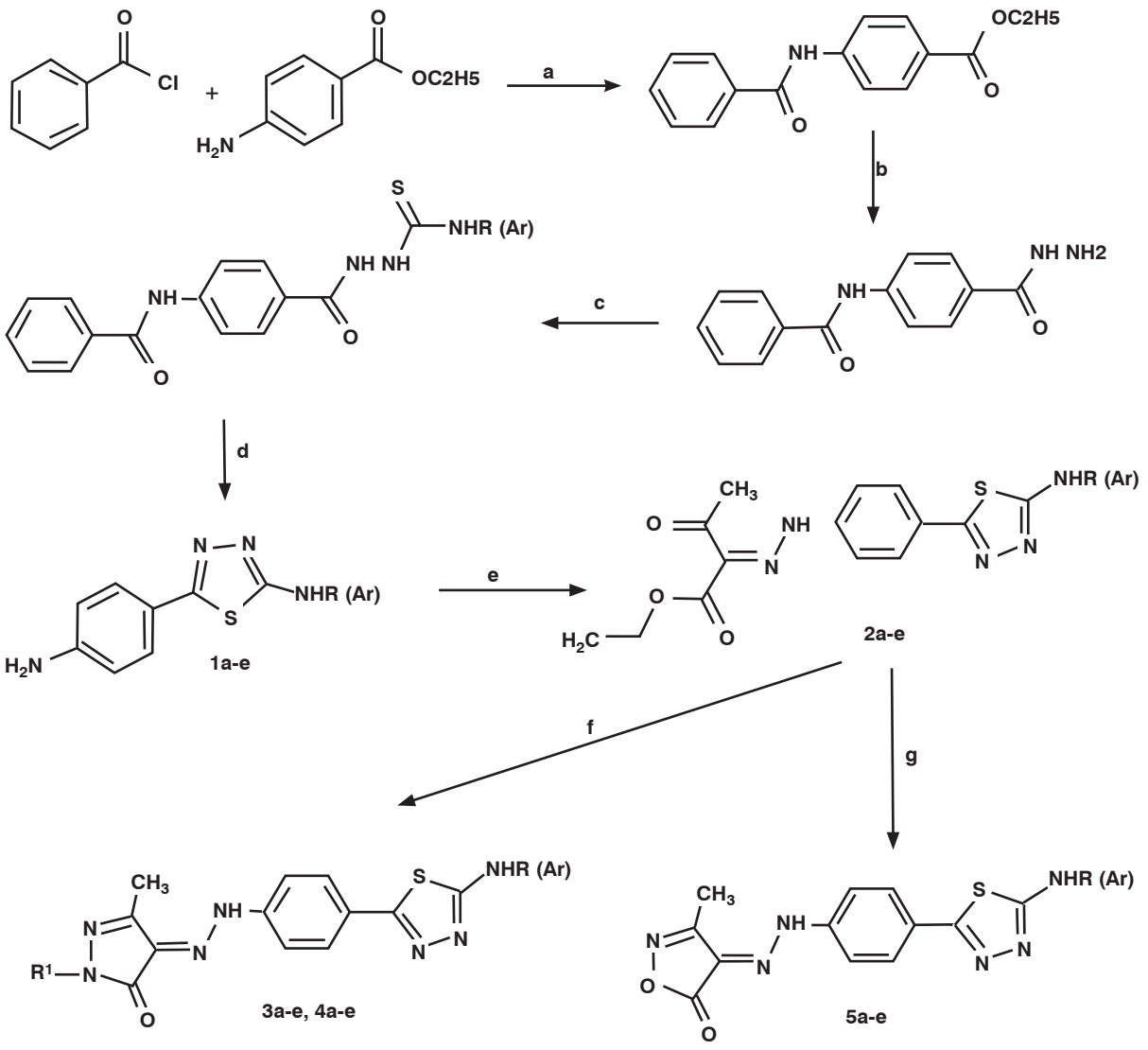
In our previous papers, we have reported that isoxazolinones (11) possess antifungal activity and 1,3,4-thiadiazoles including compounds, isoxazolinones and hydrazones (7-9,26-28) possess antitubercular and cytotoxic activities. Pyrazolinones (6) which are isosters of isoxazolidinones have also shown antitubercular activity. We incorporated different five membered ring systems for investigation of their antitubercular activity as a part of our ongoing researches on antibacterial and antitubercular activity.

RESULTS AND DISCUSSION

Structure Determination

The synthesis of hydrazone derivatives **2a-e** are been accomplished as outlined in **Scheme 1** starting from 2-(4-aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles (**1a-e**) that their multistep synthesis were reported previously by Karakuş et al (7, 26). By refluxing the intermediates ethyl 2-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-3-oxobutirates **2a-e** with hydrazine hydrate and phenyl hydrazine in glacial acetic acid, 1-(non-substituted/phenyl-3-methyl-4-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl) phenylhydrazo-no]-2-pyrazolin-5-ones **3a-e, 4a-e** were gained. 3-Methyl-4-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenylhydrazono]-5-isoxazolones **5a-e** were obtained by the reaction of **2a-e** with hydroxylamine HCl at he presence of sodium acetate in ethanolic medium.

The structures of the synthesized compounds were determined by UV, IR, ^{1}H-NMR and mass spectroscopy.



R(Ar): ethyl, cyclohexyl, phenyl, 4-chlorophenyl, benzyl; **R1:** hydrogen, phenyl; **a:** ether; **b:** hydrazin hydrate; **c:** alkyl/arylisothiocyanate; **d**: 50% sulphuric acid; **e**: sodium nitrit, hydrochloric acid, ethyl acetoacetate; **f**: hydrazin hydrate, phenyl hydrazin; **g**: hydroxyl amin hydrochlorid.

SCHEME 1. The synthesis of 2-pyrazoline-5-one and 2-isoxazoline-5-one derivatives.

In the IR spectra, the absorptions carbonyl group for **2a-e** were observed at 1712-1690 cm^{-1} (ester) and 1683-1658 cm^{-1} (ketone) regions. The ^{1}H-NMR spectra indicated the chemical shift of hydrazone NH protons between 11.52-11.63 and 13.90-14.12 ppm in the form of two singlet peaks by the reason of geometric isomerism. Strong deshielding of these protons can be explained by hydrogen bond formation (6).

The pyrazolinone carbonyl groups of compounds **3a-e, 4a-e** and isoxazolone carbonyl groups of **5a-e** were observed between 1660-1673 cm^{-1} and 1709-1717 cm^{-1} regions, respectively. The broad singlet signals that were attributed to the hydrazone N-H of **3a-e** and **4a-e** were observed at 13.30-13.84 ppm. The pyrazolinone N-H of **3a-e** were determined between 11.46-11.64 ppm (6). The protons of the methyl group attached to the 5-isoxazolone ring and hydrazone NH protons were observed as singlets at 2.13-2.27 ppm and 12.40-12.69 ppm respectively (28). M+1 peaks were obtained in the mass spectra of all novel compounds.

Antituberculosis Activity

All novel compounds except for **4a, 4c** and **4e** were tested for in vitro anti-tuberculosis activity against *M. tuberculosis* H37Rv at 6.25 μg/mL concentration. Rifampicin was used as the standard in the antimycobacterial assays. 2-(4-Aminophenyl)-5-alkyl/arylamino-1,3,4-thiadiazoles (**1a-e**) that their coupling products with acetylacetone were tested against *M. tuberculosis* H37Rv at 6.25 μg/mL previously by Karakuş et al (7) and it was declared that they demonstrated inhibition 16-57% and 0-39% respectively whereas the coupling products (compounds **2a-e**) of the same intermediate (**1a-e**) with ethyl acetoacetate exhibited 52-86% inhibition. Compound **2b** which has a cyclohexyl moiety attached to the amino group of 1,3,4-thiadiazole ring was the most active compound with 86%

TABLE 1. Antituberculer activity of 2-pyrazoline-5-one and 5-isoxazoline derivatives.

Compound	MIC (μg/ml)	inhibition (%)	Compound	MIC (μg/ml)	inhibition (%)
2a	>6.25	63	3e	>6.25	50
2b	>6.25	86	4b	>6.25	53
2c	>6.25	52	4d	>6.25	68
2d	>6.25	72	5a	>6.25	62
2e	>6.25	76	5b	>6.25	87
3a	>6.25	58	5c	>6.25	65
3b	>6.25	48	5d	>6.25	82
3c	>6.25	59	5e	>6.25	58
3d	>6.25	52			

inhibition against *M. tuberculosis* H37Rv. While compound **2c** with a phenyl moiety attached to the amino group of 1,3,4-thiadiazole ring showed 52% inhibition against *M. tuberculosis* H37Rv, a higher inhibition percentage was evaluated with the compound carrying 4-chlorophenyl moiety in stead of phenyl moiety. 1-(Nonsubstituted/phenyl-3-methyl-4-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenylhydrazono]-2-pyrazolin-5-ones **3a-e, 4a-e** except for **4a, 4c, 4e** demonstrated 48-68% inhibition against *M. tuberculosis* H37Rv at 6.25 μg/mL (Table 1). However, 3-methyl-1-phenyl-4-[4-(1-metil-1,2,4-triazolin-2(3H)-thione-2-yl)phenylhydrazono]-2-pyrazoline-5-one (8) was expanded not to have inhibition against *M. tuberculosis* H37 Rv, compounds **3d,** and **4d** showed 52% and 68% inhibition respectively and the consequence is that 1,3,4-thiadiazole ring is the source of antitubercular activity. 3-Methyl-4-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-5-isoxa-zolones **5a-e** were completely active against *M. tuberculosis* H37Rv at 6.25 μg/mL with inhibition of 58-87% and compound **5b** having cyclohexyl moiety could be elected easily from its' series with the highest inhibition value 87%.

EXPERIMENTAL

General Procedures

All solvents and reagents were obtained from commercial sources and used without purification. All melting points were determined using Buchi 530 melting point apparatus. Elemental analysis were obtained using Leco CHNS-932 and consistent with the assigned structures. Ultraviolet spectra of all compounds were recorded on Schimadzu UV 2100 S at the concentration of 0.01 mg/ml and expressed in λ_{max} (nm). Infrared spectra were recorded on Perkin Elmer 1600 and expressed in wavenumber (cm^{-1}). NMR spectra were recorded on Bruker AVANCE-DPX 400 and Mercury-VX 400 BB at 600 MHz for ^{1}H-NMR and the chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) using appropriate solvents. Mass spectra were obtained by using Fisons Instruments VG and Platform II LS-MS. The liquid chromatographic system consists of an Agilent technologies 1100 series instrument equipped with a quaternary solvent delivery system and a model Agilent 1100 series diode array detector. A Rheodyne syringe loading sample injector with a 50 μl sample loop was used for the injection of the analytes. Chromatographic data were collected and processed using Agilent Chemstataion Plus software. The seperation were performed at ambient temperature by using a reversed phase Waters Novapak C18 (3.9x150 mm, 5 μm particle size) column. All experiments were employed in isocratic mode. The mobil phase was prepared by mixing acetonitrile and bidistilled water (50: 50 v/v) and filtered through a 0.45 μm membrane and degassed by ultrasonication, prior to use. Solvent delivery was employed at a flow rate of 1 $ml.min^{-1}$. Detection of the analytes were carried out at 254 nm.

Synthesis

Compounds **(1a-e)** were prepared according to the literature (7,29-31). Ethyl 2-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-3-oxobutirates **2a-e** were synthesized by the reactions of diazonium salts of compounds **1a-e** with ethyl acetoacetate according to the literature methods (32). The coupling products **2a-e** were refluxed with hydrazine hydrate and phenyl hydrazine in glacial acetic acid for the synthesis of 1-(non-substituted/phenyl-3-methyl-4-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenylhydrazo-no]-2-pyrazolin-5-ones **3a-e** and **4a-e.** 3-Methyl-4-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenylhydrazono]-5-isoxazolones **5a-e** were gained by the reaction of coupling products **2a-e** with hydroxylamine HCl at he presence of sodium acetate in ethanolic medium.

Ethyl 2-[4-(5-ethylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-3-oxobutirate 2a

Yield: 62.34%; m.p.: 135-138°C; HPLC t_R (min.): 3.42. Anal. Calc. for $C_{16}H_{19}N_5O_3S$: C, 53.17; H, 5.30; N, 19.38; S, 8.87. Found: C, 53.67; H, 5.34; N, 18.38; S, 8.47; UV (ethanol) λ_{max}: 384, 264 nm; IR (KBr) [cm^{-1}]: 3158 (NH); 1703 (C=O ester), 1658 (C=O ketone), 1625, 1603, 1580, 1507 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.06-1.29 (m, 6H, OCH_2CH_3 and $NHCH_2CH_3$), 2.42 (s, 3H, $COCH_3$), 3.62-3.71 (m, 2H, $NHCH_2CH_3$), 4.33 (q, 2H, OCH_2CH_3), 7.51 (d, J=8.7 Hz, 2H, Ar H), 7.78 (d, J=8.7 Hz, 2H, Ar H), 7.89 (t, 1H, NH), 11.62 & 14.12 (2s, 1H, =C-N-NH); MS (CI): m/z 362 [M^++1], 348, 316, 289, 288, 261, 247, 220, 219.

Ethyl 2-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-3-oxobutirate 2b

Yield: 68.74%; m.p.: 175-178°C; HPLC t_R (min.): 8.79. Anal. Calc. for $C_{20}H_{25}N_5O_3S$: C, 57.81; H, 6.06; N, 16.85; S, 7.72. Found: C, 57.67; H, 5.95; N, 16.23; S, 7.53; UV (ethanol) λ_{max}: 385, 264 nm; IR (KBr) [cm^{-1}]: 3172 (NH), 1698(C=O ester), 1662 (C=O ketone), 1622, 1576, 1524, 1452 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.14-1.42 (m, 10H, CH_2 protons), 1.75 (t, 3H, OCH_2CH_3), 3.63 (s,1H, cyclohexyl CH), 4.03 (q, 2H, OCH_2CH_3), 7.51 (d, J=8.6 Hz, 2H, Ar H), 7.76 (d, J=8.6 Hz, 2H, Ar H), 7.85 (d, 1H, NH), 11.62 & 14.12 (2s, 1H, =C-N-NH); MS (CI): m/z 416 [M^++1], 402, 370, 344, 301, 274, 273.

Ethyl 2-[4-(5-phenylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-3-oxobutirate 2c

Yield: 66.48%; m.p.: 209-211°C; HPLC t_R (min.): 6.57. Anal. Calc. for $C_{20}H_{19}N_5O_3S$: C, 58.67; H, 4.68; N, 17.10; S, 7.83. Found: C, 57.88; H, 4.43; N, 16.65; S, 7.66; UV (ethanol) λ_{max} : 388, 273 nm; IR (KBr) [cm^{-1}]: 3197 & 3143 (NH), 1690 (C=O ester), 1664 (C=O ketone), 1619, 1603, 1570, 1501 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.31 (t, 3H, OCH_2CH_3), 2.42 (s, 3H, $COCH_3$), 4.34 (q, 2H, OCH_2CH_3), 7.03 (t, 1H, Ar H), 7.37 (t, 2H, Ar H), 7.56 (d, J=8.7 Hz, 2H, Ar H), 7.66 (d, 2H, Ar H), 7.89 (d, J=8.7 Hz, 2H, Ar H), 10.49 (s,1H, NH), 11.63 & 14.10 (2s, 1H, =C-N-NH); MS (CI): m/z 410 [M^++1], 396, 366,364, 341, 336, 313, 295, 269, 268, 261, 260.

Ethyl 2-[4-(5-(4-chlorophenyl)amino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-3-oxobutirate 2d

Yield: 61.05%; m.p.: 210-212°C; HPLC t_R (min.): 3.54. Anal. Calc. for $C_{20}H_{18}ClN_5O_3S$: C, 54.11; H, 4.09; N, 15.78; S, 7.22. Found: C, 53.92; H, 3.98; N, 15.43; S, 7.15; UV (ethanol) λ_{max} : 379, 277 nm; IR (KBr) [cm^{-1}]: 3128 (NH), 1712 (C=O ester), 1683 (C=O ketone), 1620, 1566, 1523, 1494 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.21 (t, 3H, OCH_2CH_3), 2.37 (s, 3H, $COCH_3$), 4.23 (q, 2H, OCH_2CH_3), 7.31 (d, J=8.8 Hz, 2H, ArH), 7.45 (d, J=8.7 Hz, 2H, Ar H), 7.60 (d, J=8.8 Hz, 2H, Ar H), 7.77 (d, J=8.7, 2H, Ar H), 10.50 (s,1H, NH), 11.52 & 13.90 (2s, 1H, =C-N-NH); MS (CI): m/z 444 [M^++1], 400, 398, 371, 370, 329, 302, 301, 287, 157.

Ethyl 2-[4-(5-benzylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-3-oxobutirate 2e

Yield: 65.09%; m.p.: 130-135°C; HPLC t_R (min.): 5.32. Anal. Calc. for $C_{21}H_{21}N_5O_3S$: C, 59.56; H, 5.00; N, 16.54; S, 7.57. Found: C, 59.62; H, 4.92; N, 15.93; S, 7.43; UV (ethanol) λ_{max} : 383, 263 nm; IR (KBr) [cm^{-1}]: 3200 (NH), 1711 (C=O ester), 1678 (C=O ketone), 1604, 1581, 1562, 1504 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.29 (t, 3H, OCH_2CH_3), 2.51 (s, 3H, $COCH_3$), 4.32 (q, 2H, OCH_2CH_3), 4.66 (s, 2H, CH_2), 7.23-7.42 (m, 5H, ArH), 7.54-7.63 (m, 3H, ArH and CH_2NH), 7.81 (d, J=8.8 Hz, 2H, ArH), 11.63 & 14.12 (2s, 1H, =C-N-NH); ^{1}H-NMR δ (ppm) (DMSO-d_6+D_2O): 1.21 (t, 3H, OCH_2CH_3), 2.20 (s, 3H, $COCH_3$), 4.40 (s, 4H, OCH_2CH_3 and CH_2 protons), 7.20-7.33 (m, 5H, ArH), 7.46 (d, J=8.8 Hz, 2H, Ar H), 7.70 (d, J=8.8 Hz, 2H, Ar H); MS (CI): m/z 424 [M^++1], 423, 350, 351, 309, 308, 283, 282.

3-Methyl-4-[4-(5-ethylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 3a

Yield: 58.12%; m.p.: 260-264 °C; HPLC t_R (min.): 2.31. Anal. Calc. for $C_{14}H_{15}N_7OS{\cdot}1/2H_2O$: C, 49.68; H, 4.77; N, 28.97; S, 9.47. Found: C, 49.24; H, 4.63; N, 29.02; S, 9.13; UV (ethanol) λ_{max} : 432, 308, 208 nm; IR (KBr) [cm^{-1}]: 3243 (NH), 1664 (C=O pyrazolone), 1606, 1588, 1550, 1446 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.11 (t, 3H, $NHCH_2CH_3$), 2.06 (s, 3H, CH_3), 4.12 (q, 2H, $NHCH_2CH_3$), 7.51 (d, J=8.8 Hz, 2H, Ar H), 7.6 (d, J=8.8 Hz, 2H, Ar H), 7.79 (t, 1H, NH), 11.46 (s, 1H, pyrazolone NH), 13.30 (bs, 1H, =C-N-NH); MS (CI): m/z 330 [M^++1], 303, 302, 222, 221, 220, 192, 178, 177, 136, 118, 103, 77.

3-Methyl-4-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 3b

Yield: 82.06%; m.p.: 252-256 °C; HPLC t_R (min.): 4.62. Anal. Calc. for $C_{18}H_{21}N_7OS$: C, 56.38; H, 5.52; N, 25.57; S, 8.36. Found: C, 56.63; H, 5.06; N, 24.89; S, 8.03; UV (ethanol) λ_{max} : 433, 309, 251, 210 nm; IR (KBr) [cm^{-1}]: 3189 (NH), 1663 (C=O pyrazolone), 1608, 1586, 1547, 1452 (C=C, C=N of); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.15-1.35 (m, 6H, cyclohexyl CH_2), 1.73 (d, J=8.3 Hz, 2H, cyclohexyl CH_2), 2.00 (d, J=9.9 Hz, 2H, cyclohexyl CH_2), 2.17 (s, 3H, CH_3), 3.54 (m, 1H, cyclohexyl CH), 7.61 (d, J=8.8 Hz, 2H, Ar H), 7.79 (d, J=8.8 Hz, 2H, Ar H), 7.89 (t, 1H, NH), 11.59 (s, 1H, pyrazolone NH),13.30 (bs, 1H, =C-N-NH); MS (CI): m/z 384 [M^++1], 303, 302, 275, 274, 192, 178, 177, 136.

3-Methyl-4-[4-(5-phenylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 3c

Yield: 66.21%; m.p.: 258-261°C; HPLC t_R (min.): 3.45. Anal. Calc. for $C_{18}H_{15}N_7OS$: C, 57.28; H, 4.01; N, 25.98; S, 8.50. Found: C, 57.12; H, 3.87; N, 25.21; S, 8.11; UV (ethanol) λ_{max} : 431, 309, 209 nm; IR (KBr) [cm^{-1}]: 3209 (NH), 1673 (C=O pyrazolone), 1649, 1587, 1551, 1446 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.18 (s, 3H, CH_3), 7.03 (t, 1H, Ar H),7.38 (t, 2H, Ar H), 7.67 (d, J=8.7 Hz, 4H, Ar H), 7.91 (d, J=8.7 Hz, 2H, Ar H), 10.55 (s, 1H, NH), 11.62 (s, 1H, pyrazolone NH), 13.31 (bs, 1H, =C-N=NH); MS (CI): m/z 378 [M^++1], 303, 302, 269, 268, 261, 247, 192.

3-Methyl-4-[4-(5-(4-chlorophenyl)amino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 3d

Yield: 54.09%; m.p.: 243-246 °C; HPLC t_R (min.): 10.19. Anal. Calc. for $C_{18}H_{14}ClN_7OS$: C, 52.49; H, 3.43; N, 23.81; S, 7.79. Found: C, 52.04; H, 3.24; N, 24.09; S, 7.07; UV (ethanol) λ_{max} : 431, 315, 254, 213 nm; IR (KBr) [cm^{-1}]: 3143 (NH), 1673 (C=O pyrazolone), 1604, 1585, 1532, 1441 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.17 (t, 3H, CH_3), 7.23 (d, J=8.6 Hz, 2H, Ar H), 7.42 (d, J=8.5 Hz, 2H, Ar H), 7.68 (d, J=8.6 Hz, 2H, ArH), 7.89 (d, J=8.5 Hz, 2H, Ar H), 10.51 (s, 1H, NH), 11.64 (s, 1H, pyrazolone NH), 13.41 (bs, 1H, =C-N=NH); MS (CI): m/z 412 [M^++1], 382, 304, 303, 302, 192.

3-Methyl-4-[4-(5-benzylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 3e

Yield: 54.09%; m.p.: 230-233 °C; HPLC t_R (min.): 2.85. Anal. Calc. for $C_{19}H_{17}N_7OS{\cdot}1/2H_2O$: C, 56.98; H, 4.53; N, 24.48; S, 8.01. Found: C, 56.43; H, 4.46; N, 25.01; S, 8.21; UV (ethanol) λ_{max} : 431, 311, 253, 210 nm; IR (KBr) [cm^{-1}]: 3172 (NH), 1660 (C=O pyrazolone), 1604, 1585, 1532, 1453 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.17 (t, 3H, CH_3), 4.55 (d, 2H, CH_2), 7.29-7.41 (m, 5H, Ar H), 7.61 (d, J=8.7 Hz, 2H, Ar H), 7.79 (d, J=8.7 Hz, 2H, Ar H), 8.46 (s, 1H, NH), 11.59 (s, 1H, pyrazolone NH), 13.35 (s, 1H, =C-N=NH); MS (CI): m/z 392 [M^++1], 349, 309, 301, 284, 283, 282, 258, 211, 192, 150, 136, 119, 92.

3-Methyl-1-phenyl-4-[4-(5-ethylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 4a

Yield: 63.86%; m.p.: 214-218 °C; HPLC t_R (min.): 16.49. Anal. Calc. for $C_{20}H_{19}N_7OS{\cdot}H_2O$: C, 56.72; H, 5.00; N, 23.15; S, 7.57. Found: C, 56.47; H, 4.24; N, 22.86; S, 7.03; UV (ethanol) λ_{max} :

422, 308, 245, 208 nm; IR (KBr) [cm^{-1}]: 3181 (NH), 1666 (C=O pyrazolone), 1599, 1575, 1549, 1448 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.22 (t, 3H, $NHCH_2CH_3$), 2.33 (s, 3H, CH_3), 3.34-3.39 (m, 2H, $NHCH_2CH_3$), 7.24 (t, 1H, ArH), 7.48 (t, 2H, ArH), 7.71 (d, J=8.7 Hz, 2H, Ar H), 7.83 (d, J=8.7 Hz, 2H, Ar H), 7.93 (t, 3H, Ar H and NH), 3.84 (s, 1H, =C-N-NH); MS (CI): m/z 406 [M^++1], 378, 220, 202, 192, 186, 174.

3-Methyl-1-phenyl-4-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl)phenylhydrazono]-2-pyrazolin-5-one 4b

Yield: 81.73%; m.p.: 234-237 °C; HPLC t_R (min.): 12.38. Anal. Calc. for $C_{24}H_{25}N_7OS{\cdot}1/2H_2O$: C, 61.52; H, 5.59; N, 20.93; S, 6.84. Found: C, 61.14; H, 5.43; N, 20.14; S, 6.12; UV (ethanol) $\lambda_{max:}$ 424, 315, 247, 204 nm; IR (KBr) [cm^{-1}]: 3181 (NH 1664 (C=O pyrazolone), 1583, 1546, 1505, 1450 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.24-1.35 (m, 6H, cyclohexyl CH_2), 1.73 (t, J=8.2 Hz, 2H, cyclohexyl CH_2), 2.01 (d, J=9.7 Hz, 2H, cyclohexzyl CH_2), 2.33 (s, 3H, CH_3), 3.55 (m, 1H, cyclohexyl CH), 7.25 (t, 1H, Ar H), 7.48 (t, 2H, Ar H), 7.72 (d, J=8.7 Hz, 2H, Ar H), 7.83 (d, J=8.7 Hz, 2H, Ar H), 7.94 (d, J=8.1 Hz, Ar H and NH), 13.74 (s, 1H, =C-N-NH); MS (CI): m/z 460 [M^++1], 379, 378, 274, 202, 192, 186, 174.

3-Methyl-1-phenyl-4-[4-(5-phenylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 4c

Yield: 72.68%; m.p.: 223-225 °C; HPLC t_R (min.): 11.23. Anal. Calc. for $C_{24}H_{19}N_7OS$: C, 63.56; H, 4.22; N, 21.62; S, 7.07. Found: C, 63.44; H, 4.03; N, 21.47; S, 6.77; UV (ethanol) $\lambda_{max:}$ 425, 324, 249, 203 nm; IR (KBr) [cm^{-1}]: 3199 (NH), 1669 (C=O pyrazolone), 1621, 1600, 1583, 1543, 1453 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.34 (s, 3H, CH_3), 7.04 (t, 1H, Ar H), 7.25 (t, 1H, Ar H), 7.38 (t, 2H, Ar H), 7.48 (t, 2H, Ar H), 7.67 (d, J=8.7 Hz, 2H, Ar H), 7.77 (d, J=8.7 Hz, 2H, Ar H), 7.93-7.96 (m, 4H, Ar H), 10.57 (s,1H, NH), 13.58 (s, 1H, =C-N-NH); MS (CI): m/z 454 [M^++1], 269, 268, 202, 192, 186, 174, 134, 133.

3-Methyl-1-phenyl-4-[4-(5-(4-chlorophenyl)amino-1,3,4-thiadiazole-2-yl)phenylhydrazono]-2-pyrazolin-5-one 4d

Yield: 57.16%; m.p.: 251-254 °C; HPLC t_R (min.): 18.52. Anal. Calc. for $C_{24}H_{18}ClN_7OS$: C, 59.07; H, 3.72; N, 20.09; S, 6.57. Found: C, 58.87; H, 3.64; N, 19.85; S, 6.63; UV (ethanol) $\lambda_{max:}$ 431, 315, 254, 213 nm; IR (KBr) [cm^{-1}]: 3188 (NH), 1660 (C=O pyrazolone), 1620, 1598, 1551, 1434 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.34 (s, 3H, CH_3), 7.24 (t, 1H, Ar H), 7.40-7.50 (m, 8H, Ar H), 7.59-7.61 (m, 2H, Ar H), 7.89-7.96 (m, 2H, Ar H), 10.66 (s, 1H, NH), 13.79 (s, 1H, =C-N-NH); MS (CI): m/z 488 [M^++1], 461, 460, 305, 302, 288, 202, 192, 186, 174.

3-Methyl-1-phenyl-4-[4-(5-benzylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-2-pyrazolin-5-one 4e

Yield: 54.09%; m.p.: 230-233 °C; HPLC t_R (min.): 10.42. Anal. Calc. for $C_{25}H_{21}N_7OS$: C, 64.22; H, 4.53; N, 20.97; S, 6.86. Found: C, 63.98; H, 4.47; N, 19.61; S, 6.63; UV (ethanol) $\lambda_{max:}$ 424, 315, 249, 206 nm; IR (KBr) [cm^{-1}]: 3174 (NH), 1664 (C=O pyrazolone), 1608, 1582, 1542, 1452 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.33 (s, 3H, CH_3), 4.53 (d, 2H, CH_2), 7.0-7.04 (m, 2H, Ar H), 7.28-7.32 (m, 8H, Ar H), 7.66-7.68 (m, 4H, Ar H), 8.43 (s,1H, NH), 13.54 (s, 1H, =C-N-NH); MS (CI): m/z 468 [M^++1], 426, 377, 376, 308, 202, 192, 186, 174.

3-Methyl-4-[4-(5-ethylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono] -5-isoxazolone 5a

Yield: 63.32%; m.p.: 232-235 °C; HPLC t_R (min.): 2.71. Anal. Calc. for $C_{14}H_{14}N_6O_2S{\cdot}1/2H_2O$: C, 49.55; H, 4.45; N, 24.76; S, 9.45. Found: C, 49.14; H, 4.34; N, 24.21; S, 9.16; UV (ethanol) $\lambda_{max:}$ 420, 308, 247 nm; IR (KBr) [cm^{-1}]: 3197 (NH), 1716 (C=O isoxazolone), 1604, 1592, 1551, 1499 (C=C, C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 1.21 (t, 3H, $NHCH_2CH_3$), 2.27 (s, 3H, CH_3), 3.35 (m, 2H, $NHCH_2CH_3$), 7.77 (d, J=8.8 Hz, 2H, Ar H), 7.84 (d, J=8.8 Hz, 2H, Ar H), 8.02 (s, 1H, NH), 12.68 (s, 1H, =C-N-NH); MS (CI): m/z 331 [M^++1], 286, 248, 247, 246, 221, 218, 192, 119, 103.

3-Methyl-4-[4-(5-cyclohexylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono] -5-isoxazolone 5b

Yield: 88.32%; m.p.: 220-224 °C; HPLC t_R (min.): 4.98. Anal. Calc. for $C_{18}H_{20}N_6O_2S$: C, 56.23; H, 5.24; N, 21.86; S, 8.34. Found: C,55.94; H, 5.04; N, 21.24; S, 7.96; UV (ethanol) $\lambda_{max:}$ 421, 310, 247 nm; IR (KBr) [cm^{-1}]: 3186 (NH), 1709 (C=O isoxazolone), 1585, 1545, 1496, 1455 (C=C, C=N); ^{1}H-NMR δ (ppm) ($CDCl_3$): 1.15-1.35 (m, 6H, cyclohexzyl CH_2), 1.73 (m, 2H, cyclohexzyl CH_2), 2.00 (d, J=9.9 Hz, 2H, cyclohexzyl CH_2), 2.13 (s, 3H, CH_3), 3.31 (s, 1H, cyclohexzyl CH), 7.55 (d, J=8.8 Hz, 2H, Ar H), 7.84 (d, J=8.8 Hz, 2H, Ar H), 10.64 (s,1H, NH), 12.69 (s, 1H, =C-N-NH); MS (CI): m/z 385 [M^++1], 340, 301, 275, 274, 150, 93, 65.

3-Methyl-4-[4-(5-ethylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono] -5-isoxazolone 5c

Yield: 58.32%; m.p.: 232-234 °C; HPLC t_R (min.): 2.44. Anal. Calc. for $C_{18}H_{14}N_6O_2S$: C, 57.13; H, 3.73; N, 22.21; S, 8.47. Found: C,56.94; H, 3.66; N, 22.08; S, 8.24; UV (ethanol) $\lambda_{max:}$ 398, 255 nm; IR (KBr) [cm^{-1}]: 3202 (NH), 1714 (C=O isoxazolone), 1617, 1601, 1550, 1451 (C=C , C=N); ^{1}H-NMR δ (ppm) (DMSO-d_6): 2.25 (s, 3H, CH_3), 7.24-7.28 (m, 1H, Ar H), 7.32-7.38 (m, 4H, Ar H), 7.72 (d, J=8.6 Hz, 2H, Ar H), 7.80 (d, J=8.6 Hz, 2H, Ar H), 8.44 (s,1H, NH), 12.56 (s, 1H, =C-N-NH); MS (CI): m/z 379 [M^++1], 336, 334, 269, 268, 254, 247, 246, 119, 118, 103, 102, 101, 64.

3-Methyl-4-[4-(5-(4-chlorophenyl)amino-1,3,4-thiadiazole-2-yl) phenylhydrazono] -5-isoxazolone 5d

Yield: 51.59%; m.p.: 213-216 °C; HPLC t_R (min.): 6.18. Anal. Calc. for $C_{18}H_{13}ClN_6O_2S$: C, 52.37; H, 3.17; N, 20.36; S, 7.77. Found: C, 52.11; H, 2.96; N, 20.14; S, 7.04; UV (ethanol) $\lambda_{max:}$ 407, 256 nm; IR (KBr) [cm^{-1}]: 3194 (NH), 1713 (C=O isoxazolone), 1622, 1601, 1547, 1495 (C=C, C=N); ^{1}H-NMR δ (ppm) ($CDCl_3$): 2.18 (s, 3H, CH_3), 7.38-7.55 (m, 8H, Ar H), 10.64 (s, 1H, NH), 12.58 (s, 1H, =C-N-NH); MS (CI): m/z 413 [M^++1], 398, 355, 354, 331, 330, 329, 316, 305, 304, 302, 288, 184.9, 152, 150, 128, 126, 118.

3-Methyl-4-[4-(5-benzylamino-1,3,4-thiadiazole-2-yl) phenylhydrazono]-5-isoxazolone 5e

Yield: 68.13%; m.p.: 206-209 °C; HPLC t_R (min.): 4.30. Anal. Calc. for $C_{19}H_{16}N_6O_2S$: C, 58.15; H, 4.11; N, 21.42; S, 8.17. Found: C, 57.83; H, 4.07; N, 21.13; S, 7.86; UV (ethanol) $\lambda_{max:}$ 414, 309, 246 nm; IR (KBr) [cm^{-1}]: 3194 (NH), 1717 (C=O isoxazolone), 1589, 1544, 1490, 1453 (C=C, C=N); ^{1}H-NMR δ (ppm)

($CDCl_3$): 2.27 (s, 3H, CH_3), 4.55 (d, 2H, CH_2), 7.26-7.30 (m, 1H, Ar H), 7.34-7.41 (m, 4H, Ar H), 7.75 (d, J=8.7 Hz, 2H, Ar H), 7.82 (d, J=8.7 Hz, 2H, ArH), 8.50 (s,1H, NH), 12.40 (s, 1H, =C-N-NH); MS (CI): m/z 393 [M^++1], 348, 309, 283, 150, 118, 91, 77, 64.

Antituberculosis Activity

Primary screen was conducted at 6.25 μg mL^{-1} against *M. tuberculosis H37Rv* in BACTEC 12B medium using the BACTEC 460 radiometric system (33). Compounds effecting <90% inhibition in the primary screen (MIC >6.25 μg mL^{-1}) were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentration (MIC) in a broth microdilution assay alamar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum.

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1,3,4-Tiyadiazol halkası içeren hidrazon, 2-pirazol-5-on ve 2-izoksazol-5-on türevi bileşiklerin sentezi ve antitüberküler aktiviteleri

ÖZET: Sübstitüe 1,3,4-tiyadiazol halkası içeren hidrazon (2a-e), 2-pirazol-5-on (3a-e, 4a-e) ve 2-izoksazol-5-on (5a-e) türevi bileşikler sentezlenmiş ve antitüberküler aktiviteleri incelenmiştir. Sentezlenen bileşikler arasında en yüksek inhibisyon % 87 ile 3-metil-4-[4-(5-siklohegzilamino-1,3,4-tiyadiazol-2-il)fenilhidrazono]-2-izoksazolin-5-on (5b) ve % 86 ile etil 2-[4-(5-siklohegzilamino-1,3,4-tiyadiazol-2-il)fenilhidrazono]-3-oksobutirat (2b) bileşiklerinde görülmüştür. Yeni önder bileşiklerin geliştirilmesi için 2b ve 5b maddeleri iyi bir başlangıç olabilir.

ANAHTAR SÖZCÜKLER: 1,3,4-tiyadiazol, 2-pirazolin-5-on, 2-isoksazolin-5-on, hidrazon and antitüberküler aktivite

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