*Available online*www.ijpras.com

International Journal of Pharmaceutical Research&Allied Sciences, 2022, 11(1):61-73
https://doi.org/10.51847/l4PTyjGZJw

Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Synthesis, Tautomerism Study, Antimicrobial Evaluation and Cytotoxicity of Some New Bis(Arylazo)-Terpyrazoles

Farag Altalbawy[1,2]*, Mohammed Alfadi[1]

[1]Department of Biological Sciences, University College of Duba, Tabuk University, Duba, KSA.
[2]National Institute of Laser Enhanced Sciences (NILES), Cairo University, Giza, Egypt.

***Email:**f_altalbawy@yahoo.com*

ABSTRACT

The chemistry of α-(arylhydrazono)-β-ketoaldehydes has recently received considerable attention and they are frequently employed as synthons in organic synthesis and their structural features are part of biologically active compounds. Thus, the present studies research describe the novel series synthesis of 3,3'-(5-methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-arylhydrazono-3-oxo-propanal) 3a-i via coupling reactions of sodium 3,3'-(5-methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(3-oxoprop-1-en-1-olate) (2) with arenediazonium chloride. Condensation of the compounds 3a-i with hydrazine hydrate afforded a new series of the bis(arylazo)-terpyrazole derivatives 5a-i as the end compounds. The newly prepared structures compounds were characterized as established on their spectroscopic spectra and rudimentary evauation. In addition, the spectral electronic absorption of compounds 5 was measured in various buffer solutions. The acidity constants pK's were calculated for the prepared series and their correlations versus Hammet substituent constants were investigated. The outcomes demonstraed that the title compound 5 exist in the bis-hydrazo form 5A. Producst 5a-i were screened in vitro for antibacterial and antitumor activity toward the HepG2 cell line.

Key words: *Pyrazoles, Enaminones, Hydrazone, Tautomerism, Antimicrobial, Anti-tumor activities*

INTRODUCTION

Pyrazole and their ter-heterocycles have been presented with a considerable observation because of their broad spectrum of antitumor agents, kinase inhibitor applied in tumor therapeutics and anti-proliferative activities against many cancer cell lines [1-4]. Additionally, several heterocyclic compounds incorporating pyrazole moiety have a wide range of biological activities toward NOS inhibitors, antibacterial, monoamine oxidase inhibitors, antiamoebic and potent antimicrobial activity [5-10]. In addition, arylazo and arylhydrazo heterocycles are depicted in significant groups of commonly observed organic dyes. They are utilized in several of broad industrial uses like inkjet inks, dispersed dyes, laser products, reprographic technology and laser printing [11-13]. Thanks to their photodynamic therapy and laser non-linear optical properties (NLO) appropriate for several purposes including optical switching and molecular photoprobe, other arylazo dispersion dyes have also gained considerable interest [14-17]. β-Enaminones have been used as an important precursors for synthesis of heterocyclic systems [18, 19]. In an attempt to establish new precursors, the preparation of which is less expensive than β-enaminones and used in such heterocyclic synthesis, it was considered interesting to examine the synthesis and responces of type β-hydroxyenones, R-CO-CH=CHOH. Given the above literatures and continued our preceding efforts within the bioactive heterocyclic synthesis, bis-heterocyclic compounds [20-25] and our previous work to synthesize and study spectrophotometrically arylazo and bis-arylazo derivatives of heterocyclic dyes [26, 27] to surve their utility as photoprobes, and for hydrogen ion

concentration sensing [28-30]. Herein, novel sodium 3,3'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis(3-oxo-propenolate) reported the synthesis (2) and its reactions with hetaryldiazonium salts to yield bis-hydrazonal for the synthesis of bis(arylazo)-terpyrazoles and utility of the physical constrains approach for determining its pK's to elucidate the more best tautomeric form *via* correlation the acid dissociation constant with Hammett substituent's constants using Hammett equation. The nine synthesized dyes were tested for their antimicrobial and anti-cancer activities toward HepG2 cell lines.

MATERIALS AND METHODS

The melting point was measured with a Griffin melting point device. The IR- spectrum was measured with a Pye Unicam SP 2000 infrared spectrophotometer using the KBr wafer technology. The EI-MS spectrum was determined using the AE1MS 902 mass spectrometer. The mass spectrum was recorded by the GCMS-Q1000-EX Shimadzu and the GCMS 5988-A HP spectrometers, and the ionization voltage was 70 eV. ^{1}H-NMR (300MHz) was measured on a Varian Gemini instrument using a chemical shifts (δ) in terms of downfield ppm from TMS as an internal standard for deuterated dimethylsulphoxide (DMSO-d_6). Electronic absorption spectra were recorded on Perkin-Elmer Lambda 40 spectrophotometer. Elemental evalutions were conducted by the Microanalytical Center of Cairo University, Giza, Egypt.

Synthesis of sodium 3,3'-(5-methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(3-oxoprop-1-en-1-olate) (2).
A mixture of 3,4-diacetylpyrazole derivative 1 (2.42 g, 10 mmol) and ethyl formate (1.48 g, 20 mmol) was fallen above a stirred dry ether (30 mL) solution containing MeONa (1.08 g, 20 mmol). The pure salt solid product obtained was filtrated, withered and used directly for further reactions.

Synthesis of 3,3'-(5-methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(3-oxo-2-(2- arylhydrazono) -propanal) derivatives (3a-i)
General procedure.
3,3'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis(3-oxoprop-1-en-1-olate) (2) (0.342 g, 1 mmol) in C_2H_5OH (30 mL) and sodium acetate trihydrate (0.276g, 2 mmol). The solution of sodium was cooled in ice bath at 0-5 °C with stirring Salt by adding a properportion of diazonium salt to the cold solution and diazotizing the appropriate aniline derivative (2 mmol) in hydrochloric acid (6M, 2 mL) with sodium nitrite (0.14 g, 2 mmol) in H_2O (4 mL) was prepared. After adding all the diazonium salt solution, the mixture was stirred for an additional 30 min. while cooling in a ice-water bath. The solution was then left in the refrigerator for 3days. The separated precipitate was filterated, washed with H_2O, dried and recrysallized from the relevants solvent to provide the corresponding products 3a-i. The prepared compounds 3a-i are shown benearth along the physical constants.

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(4-methoxyphenyl)-hydrazono-3-oxopropanal) (3a).
Yield (78%), mp 164-166 °C (EtOH); IR (KBr) ν_{max}: 3415 (NH), 3027, 2918 (C-H), 1725, 1688 (2C=O), 1025 (C-O-C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H, CH_3), 3.73 (s, 6H, 2OCH_3), 7.26-7.89 (m, 13H, ArH), 10.33 (s, 2H, 2CHO), 12.81 (s, 2H, 2NH); MS m/z (%): 566 (M^+, 1), 340 (18) 312 (32), 297 (48), 257 (100), 240 (30), 148 (40), 144 (21), 132, (10), 122, (15),116 (18), 103 (25), 91 (67), 76 (55), 51 (46). Anal. Calcd for $C_{30}H_{26}N_6O_6$ (566.56): C, 63.60; H, 4.63; N, 14.83. Found: C, 63.72; H, 4.50; N, 14.70%.

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(4-methylphenyl)hydrazono-3-oxopropanal) (3b).
Yield (82%), mp 152-154 °C (EtOH); IR (KBr) ν_{max}: 3410 (NH), 3019, 2935 (C-H), 1731, 1678 (2C=O), 1022 (C-O-C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.22 (s, 3H, CH_3), 2.45 (s, 6H, 2CH_3), 7.22–7.75 (m, 13H, ArH), 10.35 (s, 2H, 2CHO), 12.87 (s, 2H, 2NH); MS m/z (%): 534 (M^+, 2), 414 (32), 296 (38), 279 (48), 227 (100), 241 (42), 149 (38), 145 (27), 122, (17), 103 (32) , 91 (77), 76 (65), 63 (44). Anal. Calcd for $C_{30}H_{26}N_6O_4$ (534.56): C, 67.40; H, 4.90; N, 15.72. Found: C, 67.51; H, 4.78; N, 15.57%.

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(3-methylphenyl)hydrazono-3-oxopropanal) (3c).
Yield (72%), mp 147-149 °C (EtOH); IR (KBr) ν_{max}: 3398 (NH), 3036, 2965 (C-H), 1711, 1668 (2C=O), cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 3H, CH_3), 2.66 (s, 6H, 2CH_3), 6.88–7.80 (m, 13H, ArH), 10.39 (s, 2H, 2CHO), 12.79 (s, 2H, 2NH); MS m/z (%): 534 (M^+, 4), 414 (21), 295 (34), 281 (42), 226 (100), 240 (52), 149 (68), 147 (41), 122 (55), 116 (12), 103 (63) , 91 (80), 76 (66). Anal. Calcd for $C_{30}H_{26}N_6O_4$ (534.56): C, 67.40; H, 4.90; N, 15.72. Found: C, 67.61; H, 4.76; N, 15.63%

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(3-oxo-2-(2-phenylhydrazono)propanal) (3d). Yield 81%; mp 123-125 °C (EtOH) (Lit. mp 123-125 °C °C [31]).

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(4-chlorophenyl)hydrazono-3-oxopropanal) (3e).
Yield (86%), mp 193-195 °C (EtOH); IR (KBr) ν_{max}: 3378 (NH), 3030, 2965 (C-H), 1710, 1688 (2C=O), cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.23 (s, 3H, CH_3), 6.58–7.56 (m, 13H, ArH), 10.32 (s, 2H, 2CHO), 12.86 (s, 2H, 2NH); MS m/z (%): 577 (M^++2, 2), 575 (M^+, 5), 440 (33), 315 (24), 295 (56), 240 (100), 149 (28), 121(53), 114 (31), 105 (73), 91 (80), 76 (85), 51 (66). Anal. Calcd for $C_{28}H_{20}Cl_2N_6O_4$ (575.40): C, 58.45; H, 3.50; N, 14.61. Found: C, 58.28; H, 3.35; N, 14.54%.

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(3-chlorophenyl)hydrazono-3-oxopropanal) (3f).
Yield (71%), mp 173-175 °C (EtOH); IR (KBr) ν_{max}: 3348 (NH), 3048, 2955 (C-H), 1711, 1668 (2C=O), cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 3H, CH_3), 6.78–7.76 (m, 13H, ArH), 10.12 (s, 2H, 2CHO), 12.95 (s, 2H, 2NH); MS m/z (%): 577 (M^++2, 3), 575 (M^+, 10), 440 (23), 315 (34), 295 (66), 241 (100), 149 (48), 132 (43), 121 (63), 115 (54), 105 (87), 91 (89), 76 (65), 51 (82). Anal. Calcd for $C_{28}H_{20}Cl_2N_6O_4$ (575.40): C, 58.45; H, 3.50; N, 14.61. Found: C, 58.36; H, 4.39; N, 14.58%.

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(4-bromophenyl)hydrazono-3-oxopropanal) (3g).
Yield (80%), mp 184-186 °C (EtOH); IR (KBr) ν_{max}: 3410 (NH), 3041, 2965 (C-H), 1717, 1648 (2C=O), cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 7.04–7.96 (m, 13H, ArH), 10.07 (s, 2H, 2CHO), 12.82 (s, 2H, 2NH); MS m/z (%): 666 (M^++2, 5) 664 (M^+, 6), 481(28), 402 (24), 387 (44), 295 (36), 242 (100), 149 (28), 132 (35), 115 (33), 105 (58), 91 (80), 76 (55), 51 (52). Anal. Calcd for $C_{28}H_{20}Br_2N_6O_4$ (664.30): C, 50.62; H, 3.03; N, 12.65. Found: C, 50.46; H, 3.00; N, 12.53%.

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(2-nitrophenyl)hydrazono-3-oxopropanal) (3h). Yield (68%), mp 204-206 °C (dioxane); IR (KBr) ν_{max}: 3401 (NH), 3037, 2975 (C-H), 1718, 1668 (2C=O), cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 7.24–8.06 (m, 13H, ArH), 10.12 (s, 2H, 2CHO), 12.90 (s, 2H, 2NH); MS m/z (%): 596 (M^+, 2), 446 (68), 402 (45), 298 (32), 241 (100), 150 (38), 122 (47), 114 (36), 105 (47), 76 (85), 51 (42). Anal. Calcd for $C_{28}H_{20}N_8O_8$ (596.50): C, 56.38; H, 3.38; N, 18.78. Found: C, 56.27; H, 3.22; N, 18.55%.

3,3'-(5-Methyl-1-phenyl-1H-pyrazole-3,4-diyl)bis(2-(4-nitrophenyl)hydrazono-3-oxopropanal) (3i).
Yield (64%), mp 264-266 °C (dioxane); IR (KBr) ν_{max}: 3431 (NH), 3035, 2955 (C-H), 1721, 1678 (2C=O), cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H, CH_3), 7.34–8.26 (m, 13H, ArH), 10.28 (s, 2H, 2CHO), 13.2 (s, 2H, 2NH); MS m/z (%): 596 (M^+, 7), 445 (77), 401 (52), 301 (22), 243 (100), 152 (39), 122, (36), 116 (22), 105 (51), 91 (66), 76 (54), 51 (81). Anal. Calcd for $C_{28}H_{20}N_8O_8$ (596.50): C, 56.38; H, 3.38; N, 18.78. Found: C, 56.52; H, 3.32; N, 18.69%.

Alternative synthesis of 3d
Carry out the same above coupling reaction using 1,1'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis(3-(dimethyl -amino)prop-2-en-1-one) (4) (0.352 g, 1 mmol) instead of 2. The precipitated product was filtrated, washed with H2O and lastly recrystallized from ethanol to provide a product proved identical in all respects (mp, mixed mp and IR spectra) with compound 3d, with those bought from coupling of 2 with PhN_2Cl but in 70% yield.

General method for synthesis of 5'-methyl-1'-phenyl-4,4''-bis(aryldiazenyl)- 1H,1'H,1''H-3,3':4',3''-terpyrazole derivatives 5a-i:
A mixture of the appropriate bis(2-arylhydrazono-3-oxo-propanal) derivatives 3a-i (1mmol) and hydrazine hydrate (2 mL) in dioxane (10 ml) was refluxed for 10-15 h. The formed solid after cooling the solution was filtered off and crtstallized from the proper solvent to give the corresponding ter-pyrazoles 5a-i. The products obtained are described below along with their analytical data.

4,4''-Bis((4-methoxyphenyl)diazenyl)-5'-methyl-1'-phenyl-1H,1'H,1''H-3,3':4',3''-terpyrazole (5a).
Yield (72%), mp 292-294 °C (DMF); IR (KBr) ν_{max}: 3419 (NH), 3025, 2918 (C-H), 1695 (C=N), 1024 (C-O-C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.75 (s, 3H, CH_3), 3.71 (s, 6H, 2OCH_3), 7.46-7.89 (m, 15H, ArH and pyrazole-H), 10.24 (s, 2H, D_2O-exchangeable, 2NH); MS m/z (%): 558 (M^+, 2), 412 (32), 380 (15) 292 (41), 226 (22),

152 (48), 137 (24), 103 (100), 91 (65), 76 (75), 51 (90). Anal. Calcd for $C_{30}H_{26}N_{10}O_2$ (558.59): C, 64.51; H, 4.69; N, 25.08. Found: C, 64.37; H, 4.53; N, 25.00%.

5'-Methyl-1'-phenyl-4,4''-bis(p-tolyldiazenyl)-1H,1'H,1''H-3,3':4',3''-terpyrazole (5b).
Yield (68%), mp 280-282 °C (dioxane); IR (KBr) ν_{max}: 3415 (NH), 3028, 2948 (C-H), 1690 (C=N), cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.41 (s, 6H, $2CH_3$), 7.32-7.72 (m, 15H, ArH and pyrazole-H), 10.17 (s, 2H, D_2O-exchangeable, 2NH). MS m/z (%): 526 (M^+, 4), 397 (21), 382 (42), 291(35), 225 (18), 159 (47), 116 (18), 103 (100), 91 (48), 76(59), 63 (38), 51 (67). Anal. Calcd for $C_{30}H_{26}N_{10}$ (526.59): C, 68.42; H, 4.98; N, 26.60. Found: C, 68.59; H, 5.10; N, 26.87%.

5'-Methyl-1'-phenyl-4,4''-bis(m-tolyldiazenyl)-1H,1'H,1''H-3,3':4',3''-terpyrazole (5c).
Yield (62%), mp 275-277 °C (DMF); IR (KBr) ν_{max}: 3412 (NH), 3025, 2966 (C-H), 1687 (C=N), cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH3), 2.39 (s, 6H, $2CH_3$), 6.88-7.59 (m, 15H, ArH and pyrazole-H), 10.02 (s, 2H, D_2O-exchangeable, 2NH); MS m/z (%): 526 (M^+, 8), 395 (34), 380 (22), 290 (44), 224 (38), 158 (77), 115 (48), 101 (100), 91 (42), 76 (61), 51 (68). Anal. Calcd for $C_{30}H_{26}N_{10}$ (526.59): C, 68.42; H, 4.98; N, 26.60. Found: C, 68.37; H, 5.03; N, 26.46%.

5'-Methyl-1'-phenyl-4,4''-bis(phenyldiazenyl)-1H,1'H,1''H-3,3':4',3''-terpyrazole (5d).
Yield 76%; mp > 300 °C (DMF) (Lit. mp > 300 °C °C [31]).

4,4''-Bis((4-chlorophenyl)diazenyl)-5'-methyl-1'-phenyl-1H,1'H,1''H-3,3':4',3''-terpyrazole (5e).
Yield (73%), mp >300 °C (DMF); IR (KBr) ν_{max}: 3426 (NH), 3028, 2958 (C-H), 1665 (C=N), cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.29 (s, 3H, CH_3), 6.92-7.78 (m, 15H, ArH and pyrazole-H), 10.11 (s, 2H, D_2O-exchangeable, 2NH); MS m/z (%): 569 (M^++2, 4), 567 (M^+, 14), 416 (21), 292 (35), 224 (36), 158 (42), 142 (23), 124 (25), 116 (48), 103 (100) , 91 (66), 76 (57), 51 (33). Anal. Calcd for $C_{28}H_{20}Cl_2N_{10}$ (567.43): C, 59.27; H, 3.55; N, 24.68. Found: C, 59.14; H, 3.42; N, 24.51%.

4,4''-Bis((3-chlorophenyl)diazenyl)-5'-methyl-1'-phenyl-1H,1'H,1''H-3,3':4',3''-terpyrazole (5f).
Yield (71%), mp > 300 °C (DMF); IR (KBr) ν_{max}: 3432 (NH), 3046, 2972 (C-H), 1685 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.31 (s, 3H, CH_3), 6.96-7.73 (m, 15H, ArH and pyrazole-H), 10.18 (s, 2H, D_2O-exchangeable, 2NH); MS m/z (%): 569 (M^++2, 5), 567 (M^+, 17), 415 (22), 290 (45), 224 (25), 159 (65), 141 (53), 131 (25), 124 (44), 116 (40), 101 (100), 91 (34), 77 (87), 51 (52). Anal. Calcd for $C_{28}H_{20}Cl_2N_{10}$ (567.43): C, 59.27; H, 3.55; N, 24.68. Found: C, 59.39; H, 3.47; N, 24.55%.

4,4''-Bis((4-bromophenyl)diazenyl)-5'-methyl-1'-phenyl-1H,1'H,1''H-3,3':4',3''-terpyrazole (5g).
Yield (76%), mp > 300 °C (DMF); IR (KBr) ν_{max}: 3443 (NH), 3029, 2978 (C-H), 1680 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 6.86-7.82 (m, 15H, ArH and pyrazole-H), 10.31 (s, 2H, D_2O-exchangeable, 2NH); MS m/z (%): 658 (M^++2, 6), 656 (M^+, 7), 464 (34), 294 (28), 226 (54), 160 (39), 141 (18), 133 (15), 126 (26), 114 (41), 105 (100), 91 (62), 76 (54), 51 (39). Anal. Calcd for $C_{28}H_{20}Br_2N_{10}$ (656.33): C, 51.24; H, 3.07; N, 21.34. Found: C, 51.32; H, 3.05; N, 21.31%.

5'-Methyl-4,4''-bis(2-nitrophenyl)diazenyl)-1'-phenyl-1H,1'H,1''H-3,3':4',3''-terpyrazole (5h).
Yield (70%), mp > 300 °C (DMF); IR (KBr) ν_{max}: 3448 (NH), 3031, 2974 (C-H), 1685 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 6.96-7.79 (m, 15H, ArH and pyrazole-H), 10.11 (s, 2H, D_2O-exchangeable, 2NH); MS m/z (%): 588 (M^+, 5), 440 (45) 291 (34), 238 (31), 223 (45) 158 (41), 144 (22), 103 (100), 91 (77), 76 (52), 51 (57). Anal. Calcd for $C_{28}H_{20}N_{12}O_4$ (588.53): C, 57.14; H, 3.43; N, 28.56. Found: C, 57.09; H, 3.30; N, 28.51%.

5'-Methyl-4,4''-bis(4-nitrophenyl)diazenyl)-1'-phenyl-1H,1'H,1''H-3,3':4',3''-terpyrazole (5i).
Yield (77%), mp > 300 °C (DMF); IR (KBr) ν_{max}: 3452 (NH), 3032, 2978 (C-H), 1688 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 7.15-7.98 (m, 15H, ArH and pyrazole-H), 10.26 (s, 2H, D_2O-exchangeable, 2NH). MS m/z (%): 588 (M^+, 10), 442 (48) 290 (65), 237 (38), 224 (49) 156 (48), 101 (100), 92 (76), 76 (22), 51 (71). Anal. Calcd for $C_{28}H_{20}N_{12}O_4$ (588.53): C, 57.14; H, 3.43; N, 28.56. Found: C, 57.22; H, 3.37; N, 28.47%.

Prepared pK's Determination of bis(arylazo)-terpyrazole derivatives 5a-i

The rial methods go along with the analysis of the dissociation constants pKa and their estimates are based on the electronic absorbance-pH data as reported earlier in the literature [32, 33].

Antimicrobial evaluation

In this analysis, the Gram-negative bacteria *PA* and Gram +ve bacteria *SP* and *ECand BS*, and the fungal *AF*, *SR*, *GC* and *CA* strains were used for examining the prepared compounds for their antimicrobial and fungicde activities. The selected species were obtained from the regional center for biotechnology and mycology of Al-Azhar University, Cairo, Egypt [34-37].

Control antibacterial and antifungal

Control antibacterial as Ampicillin and Gentamicin and antifungal as Amphotericin B were purchased from Sigma-Aldrich (St Louis, MO, USA) and used as a comparable references

Anti-Tumor evaluation

The human liver cancer (HepG-2) cell line was acquired from the American Type Culture Collection (ATCC, Rockville, Marylan). Cells were cultured in RPMI-1640 medium and added with 10% inactivated fetal bovin serum and 50µg/mL gentamicin. The cells were maintained at 37°C in a humidified atmosphere 0f 5% CO_2 and were sub-cultured 2 to 3 times a week.

In the anti-cacer assays, the tumor cell lines were suspended in the medium at a concentration of $5x10^4$ cell/well on corning 96-well corning plates (6 repeats) achieving 8 concentrations for every compound. Six vehicle controls using medium or 0.5% DMSO were performed on every 96 well plates as controls. After 24 hours of incubating, the amount live cells was measured by MTT assay. Concisely, the medium is removed from the 96 well plates and replaced with 100 µL fresh RPMI 1640 medium without phenol red, followed by 10 µL, 12 µL MTT stock (5 mg of MTT in 1 mL of PBS) in each well comprising unprocessed controls. The 96-well plates was then incubated at 37°c and 5% CO_2 for 4 hours. An 85µL of the medium was removed from the wells, and 50µL of DMSO was added to every well mixed thoroughly with a pipette, and incubated at 37°c for 10 minutes. Subsequently, amicroplate reader (Sunrise, TECAN, Inc, USA) was used to measure the optical density at 590nm, the live cells count was measured, and the viability was (1-(ODt/ODc)) x 100% calculated as ODt is the average optical density of untreated cells. Plot the relationship between drug concentration and viable cells to acquire the stable curve for every tumor cell line after treatment with the indicated compound. The 50% inhibitory concentration (IC_{50}), which necessary to produce a toxic effects in 50% of intact cells, is a dose-response curve plot for every concentration with Graphed Prism software (San Diego, CA. USA) was decided from [38-41].

RESULTS AND DISCUSSION

Chemistry

The starting sodium 3,3'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl)bis(3-oxoprop-1- en-1-olate) (2) was prepared *via* a reaction of 1-phenyl-3,4-diacetyl-5-methylpyrazole (1) [11] with two equivalents of ethyl formate in etheric CH_3NaO solution. The sodium reactivity (pyrazole-diyl)bis(3-oxopropenolate) derivative 2 was allowed to react with arenediazonium salts to synthesize new series of *bis*-arylhydrazone derivatives carrying pyrazole moiety. Thus, couplling compound 2 with two moles of each of arenediazonium chlorides in ethanolic sodium acetate trihydrate afforded the corresponding *bis*-arylhydrazone coupling products 3a-i in decent to perfect yields (64-86%) **(Figure 1)**.

The compound 3a-i structure was established based on spectral data and micro-analytical analysis (Ms, IR and ^{1}H NMR). The Ms spectra of all compounds 3 revealed, in each case, a respective molecular ion peak at the correct formula molecular weight. The infrared absorption spectra of hydrazones 3a-i showed in each derivative one band at 3,348-3,431 cm^{-1} for NH stretching absorptions and two strong absorption bands observed at 1,648-1668 and 1,710-1,731 cm^{-1} for the two stretching vibrations C=O groups. Their spectrum of ^{1}H NMR revealed in each case the expected singlet and multiplieds signals of the methyl and aromatic protons at δ 2.22-2.38 and 6.58-8.26 ppm, respectively. In addition these bands also showed two singlet bands one of them in the regions δ 10.02-10.39 assignable to -CHO proton and the other singlet in the region δ 12.79-13.2 ppm, due to hydrazone NH group. These δ values agree with those of the *Z*-isomers of ($Ar_1COC(CHO)=NNHAr_2$) which were previously reported in the literature to show their aldehydic proton signals in the region δ 9.50-9.63 and

hydrazone proton band in the region δ 11.85-12.78 ppm [33, 42]. The ^{1}H-NMR spectrum of the E-isomers, as outlined in **Figure 2** of the 3-aryl-3-oxo-2-arylhydrazonopropanals revealed the two singlet signals at δ 9.96-10.17 and δ 13.9-14.35 ppm for their aldehydic and hydrazone protons, respectively [31, 42]. This indicated that product 3 is present only in the *Z*-isomers **(Figure 2)**.

In addition, to provide conclusive evidence of the assigned structure 3a-i, prepared by the condensation reaction of bisenaminose 4 [43] (compound 1 and 2 equivalents of dimethylformamide dimethylacetal (DMF-DMA) with benzene diazonium chloride in ethanol) coupling reaction was performed. the sodium acetate trihydrate presence yielded a product that was found to be comparable to 3a at all points (IR, MS, mp. and mixed mp.) **(Figure 1)**.

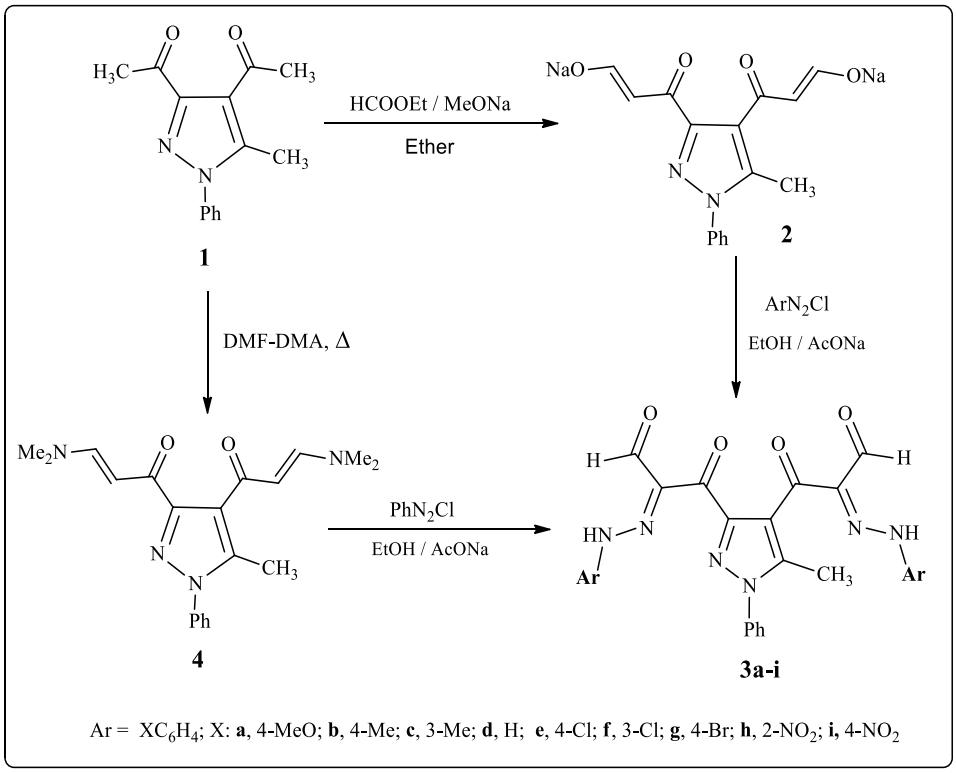


Figure 1. Synthesis of *bis*-arylhydrazonopropanal derivatives 3a-i

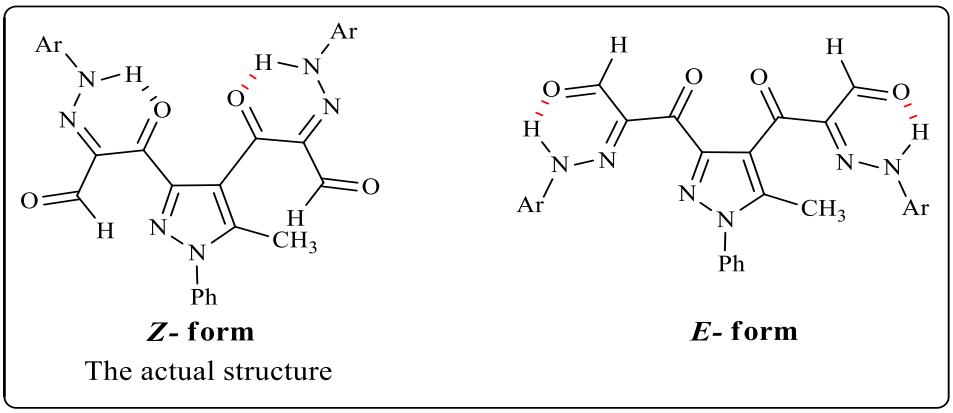


Figure 2. Isomers of *bis*-arylhydrazonopyrazolederivatives 3a-i

Next, refluxing of the *bis*-arylhydrazone derivatives 3a-i with hydrazine hydrate in dioxane yielded the corresponding single products, 5a-i (as confirmed by the TLC spectrum of the crude products), as depicted in **Figure 3**. The assigned corrected structure of 5'-methyl-1'-phenyl-4,4"-bis(aryldiazenyl)-1*H*,1'*H*,1''*H*-3,3':4',3"-terpyrazole derivatives 5a-i were elucidated by their elemental analyses, ^{1}HNMR, infrared absorptions and mass fragmentations spectral data. The IR absorption bands of hydrazone derivatives 5a-i (See Experimental section) exhibited an absorption peak in each derivative in the ranges ν_{max} 3412-3452 cm^{-1} due to the stretching

hydrazone NH group in addition to the two expected peaks of CH aliphatic and C=N stretching. The mass fragmentation spectra of all terpyrazole 5a-i showed the molecular ion peak (M^+) of each derivative with the correct formula weight (M^+) for derivative (See Experimental section). The resulting spectroscopic analysis persistent with the designated structurales formula 5a-i, but differentiates between the two probable tautomeric types is incapable, the *bis*-arylhydrazone and *bis*-arylazo types 5A an 5B, respectively. UV-V spectrum was measured at various pH solutions To confirm the exact tautomeric structure of product 5.

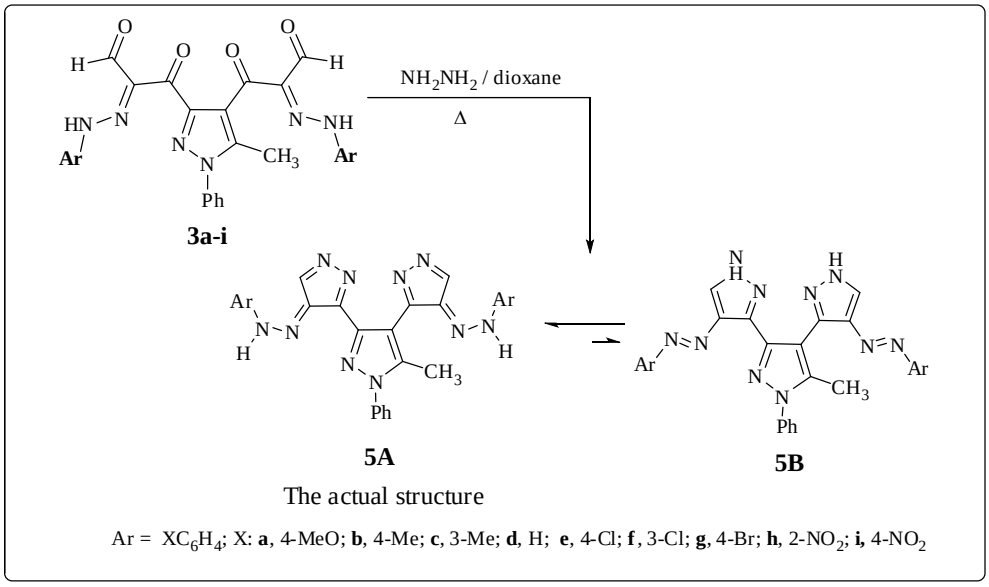


Figure 3. Synthesis of *bis*-arylazo-terpyrazole derivatives 5a-i.

Determination of pKa's and actual tautomeric form of the products 5a-i

The UV-Vis absorption spectra of terpyrazole derivatives 5a-i in ethanol outlined, in every scenario, two traits peaks, one in the region of 414-395 nm and the second absorption within the 337-313 nm range **(Table 1)**. These UV-Vis data are typical of those of arylhydrazone chromophore [22, 26]. So it can be concluded that the studied dyes 5 are present in solution as one tautomeric form, namely the bis-arylhydrazone tautomeric form 5A.

To dispense additional evidence 0f the assignment of arylhydrazone 5A to studied products 5a-i, the pKa constants of the prepared series were calculated spectrophotometrically. A typical UV-Vis absorption spectrum of 5d buffers of various pH values is indicatwd **(Figure 4)**. From the absorbance-pH spectra, the value of the acid dissociation constants pKa was determined [44, 45] using equation (1):

$$pK=pH_i+\log(A_b-A_i)/(A_i-A_a) \quad (1)$$

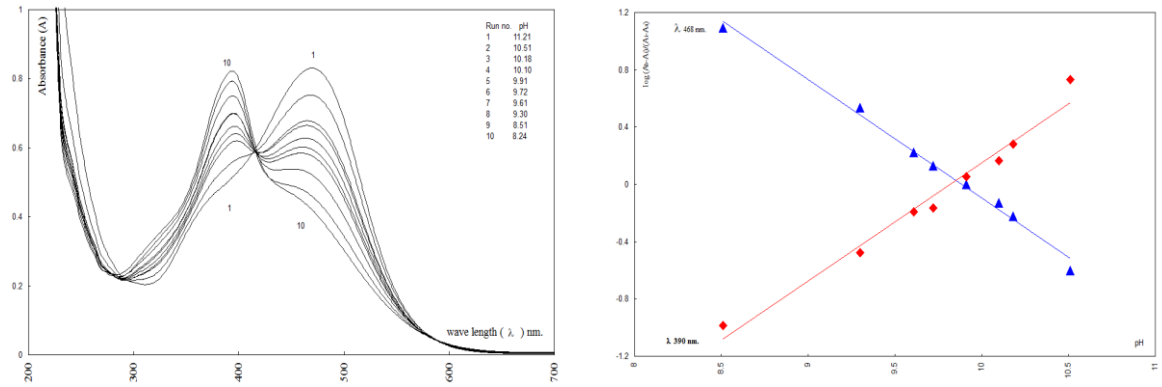
Where A_i is the absorbance of the test solution at pH_i, and A_b and A_a are the absorbance values of a strongly base and acidic solution [40]. The dissociation acidity constants pKa values calculated for the prepared products 5a-i are outlined in **(Table 1)**. Equations 2 and 3 comparable to the straight lines acquired are:

$$pKa=9.90-1.4\ \sigma_x\ ,^{r^2} = 0.94,\ s = \pm 0.09) \quad (2)$$

$$pKa=9.91-1.4\ \sigma_x^-\ ,^{r^2} = 0.98,\ s = \pm 0.07) \quad (3)$$

Where r and s are the correlation coefficient and standard deviation, respectively. The pKa data of compounds 5a-i from the values of r and s seem to be preferable associated with the enhanced Hammett substituent constant σ^- rather than the Hammett substituent constant σ are shown in **(Figure 5)**. Such excellent correlations with Hammett substituent constant indicate that the studied compounds 5a-i exists in the bis-hydrazone Tautomeric form 5A in solution. This is because if the prepared compounds 5 exist as equilibrium mixture of 5A and 5B, as outlined in **(Figure 3)**, no linear relationship is observed between pKa and σ^-x. In addition, the reaction rate ρ =

1.09 appears to indulge the *bis*-hydrazone form 5A as it is in decentagreement with previously described values for comparable bis-hydrazones rather than bis-arylazo derivatives [32, 46].



(a) (b)

Figure 4. (a) Electronic Absorption Spectra of compound 5d, in solution of different pH values (1:4 (v/v) dioxane-water) at 27 ^{0}C and μ=0.10.; (b) Correlation of $\log(A_b - A_i)/(A_i - A_a)$ at two maximum electronic absorption wave lengths with pH values of compound 5d.

Table 1. UV/Vis Spectra and acid dissociation constants pK of *bis*-arylhydrazonopyrazole derivatives 5a-i.

Compound No.	λ_{max} nm (EtOH) (log ε)	σ_X	σ^-_X	pK	λ_{max}a	λ_{max}b	Δν cm^{-1}	pK*
4a	401(4.12), 321(4.22)	−0.27	−0.27	10.29	393	475	4392	1.06
4b	397(4.06), 325(4.15)	−0.17	−0.17	10.15	392	469	4188	1.35
4c	395(4.00), 323(4.25)	−0.07	−0.07	9.90	388	467	4359	0.78
4d	392(4.15),313(4.10)	0.00	0.00	9.85	390	468	4273	0.87
4e	400(4.42), 325(4.40)	0.23	0.23	9.75	396	479	4375	0.56
4f	406(4.10), 337(4.12)	0.37	0.37	9.49	392	470	4233	0.60
Dg	409(4.40),330(4.45)	0.71	0.71	9.03	400	480	4166	0.28
4h	402(4.41), 335(4.33)	0.5	0.84	9.04	400	483	4296	0.02
4i	414(3.39), 337(3.54)	0.78	1.28	8.56	400	485	4381	-0.64

Note. [a] In acid medium; [b] in alkaline medium; ± s = 0.05.

Next, the acidity constants in excited state pK*'s of the studied compounds 5a-i were determined using the Forester energy cycle [28, 29]. According to this energy cycle the equation 4:

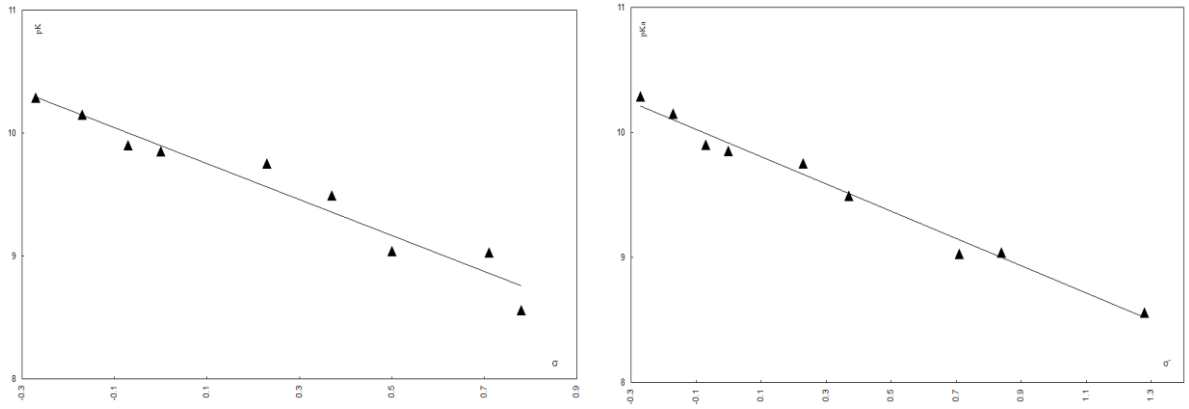
$$pK^* = pK + (\Delta\nu / 0.625/T) \quad (4)$$

Here, pK and pK* are the acid dissociation constants in the ground state and the excited states, respectively and Δν the frequency difference in cm^{-1} units between the maximum λ_{max} absorption values. Any compound in acidic and alkaline media. The computations outcomes of are shown in **(Table 1)**. The interrelation between these pK* data and σx is shown in **(Figure 5)**. The linear equations 5 and 6 equivalent to such interrelations are:

$$pK^* = 0.87 - 1.40\ \sigma_x, r^2 = 0.81, s = \pm 0.28 \quad (5)$$

$$pK^* = 0.90\text{-}1.10\ \sigma_x^-,\ r^2 = 0.93,\ s = \pm 0.17 \quad (6)$$

Such linear equations indicate that studied series 5a-i has also predominantly the *bis*-hydrazone tautomeric form in their excited states [47, 48].



a) b)

Figure 5. Correlation of pKa *bis*-arylhydrazonopyrazole derivatives 5a-i (a) with Hammett substituent constant, σ_X. (b) with enhanced Hammett substituent constant, σ^-_X.

Antimicrobial evaluation

In recent study, the title-synthesized terpyrazole derivatives 5a-i were assessed for in vitro antimicrobial activity against Gram -ve bacteria such as E coli (EC), Pseudomonas aeruginosa (PA) and Gram-positive bacteria eg. Bacillis subtilis (BS), Streptococcus pneumoniae (SP) and for their in vitro fungicid activity towards Syncephalastrum racemosum (SR), Aspergillus fumigatus (AF), Candida albicans (CA), and Geotricum candidum (GC) fungal strains. Under similar conditions, the bactericides Gentamicin and Ampicillin and the antifungal Amphotericin B wer utilized as guide lines to determine the effectiveness of the compounds evaluated. The diameter of the inhibition zone (IZD) was used as the criterion for the antibacterial activity using diffusion technology **(Table 2)**.

Table 2. Antimicrobial activities of the synthesized bis(arylazo)-terpyrazoless (5a-i).

Comp.	Inhibition zone diameter (cm)							
	Gram (+)		Gram (−)		Fungi			
Standard drugs	*(SP)*	*(BS)*	*(PA)*	*(EC)*	*(AF)*	*(SR)*	*(GC)*	*(CA)*
	23.8 ± 0.2	32.4 ± 0.3	17.3 ± 0.1	19.9 ± 0.3	23.7 ± 0.2	19.7 ± 0.2	28.7 ± 0.2	25.4 ± 0.1
5a	14.3 ± 0.3	14.9 ± 0.3	NA	NA	15.2± 0.5	11.3 ± 0.3	11.7 ± 0.5	NA
5b	12.1 ± 0.4	16.3 ± 0.3	NA	NA	16.4± 0.3	11.6 ± 0.3	12.0 ± 0.4	NA
5c	12.4 ± 0.3	15.2 ± 0.4	NA	NA	15.7 ± 0.3	12.1 ± 0.2	12.3 ± 0.3	NA
5d	15.3 ± 0.3	17.6 ± 0.4	10.1 ± 0.3	8.2 ± 0.2	15.6 ± 0.6	12.7 ± 0.3	11.4 ± 0.4	13.6 ± 0.6
5e	19.1 ± 0.4	22.8 ± 0.3	13.1 ± 0.4	20.3 ± 0.1	20.2 ± 0.6	16.4 ± 0.6	22.4 ± 0.6	17.9 ± 0.4
5f	17.8 ± 0.5	20.4 ± 0.3	12.1 ± 0.3	19.1 ± 0.1	17.3 ± 0.4	13.2 ± 0.3	19.0 ± 0.6	17.3 ± 0.4
5g	18.7 ± 0.6	20.7 ± 0.4	14.1 ± 0.4	17.3 ± 0.1	18.9 ± 0.6	15.4 ± 0.3	20.7 ± 0.3	19.4 ± 0.3
5h	14.8 ± 0.6	15.2 ± 0.7	10.3 ± 0.4	14.6 ± 0.3	14.1 ± 0.5	14.0 ± 0.4	13.7 ± 0.5	13.7 ± 0.5
5i	16.9 ± 0.4	19.8 ± 0.6	11.1 ± 0.3	15.7 ± 0.3	15.3 ± 0.6	14.4 ± 0.4	19.5 ± 0.4	16.2 ± 0.3

Note. Data are expressed in the form of mean ± SD. Mean zone of inhibition in mm ± standard deviation beyond well diameter; (6 mm) produced on a range of environmental and clinically pathogenic microorganism using (5 mg/mL) concentration of tested sample (100 µL was tested).

The outcomes summarized in **(Table 2)** indicated: SP and BS are sensitive to all examined compounds 5a-i; furthermore, EC and PA are sensitive to compounds 5d-i. All tested compounds except compounds 5a-c exhibited antifungal activity against the four tested fungal strains SR, AF, CA and GC. Whereas, compound 5a-c showed no the antifungal activity against the one fungal species CA. The high activity of all compounds tested is due to the pharmacological active presence of ter-pyrazole moietist in all compounds 5a-i. The inactivity of the ter-pyrazole derivatives 5a-c towards the tested CA and Gram-negative bacteria is caused by the presence ofelectron-donor groups.

Anti-tumoractivity

The antitumor activity of the newly synthesized hydrazones compounds **5a-i** was decided toward the liver cancer cell line HEPG2, using doxorubicin as a reference drug. The data created was used to plot a dose-response curve in which the concentration of the test compounds needed to kill 50% of the cell population (μM) was determined. The cytotoxic activity was presented as the mean IC_{50} of three independent experiments **(Table 3)**, and the outcomes indicated that:

Table 3. The in vitro inhibitory activity of tested compounds expressed as IC_{50} values (μM) ± standard deviation from five replicates against HEPG-2 cell line.

Compound No.	Ar	IC_{50} (μM)	Compound No.	Ar	IC_{50} (μM)
5a	4-$MeOC_6H_4$	11.7± 0.19	**5f**	3-ClC_6H_4	6.32 ± 0.24
5b	4-MeC_6H_4	12.48± 0.26	**5g**	4-BrC_6H_4	2.15 ± 0.12
5c	3-MeC_6H_4	14.34± 0.21	**5h**	2-$NO_2C_6H_4$	4.4 ± 0.15
5d	C_6H_5	14.45± 0.18	**5i**	4-$NO_2C_6H_4$	2.6 ± 0.09
5e	4-ClC_6H_4	4.35 ± 0.16	**Doxorubicin**	-	± 0.18

All compounds tested showed concentration-dependent inhibitory activity to the cancer cell lines. The lower IC_{50} values of the chosen compounds revealed that, higher concentrations can be used for stronger antitumor activity. The outcomes are shown in **(Table 3)** and indicate that: Shier *et al*. *In vitro* inhibitory activity of test [49]. Compound 5e and compounds 5g-i have strong *in vitro* inhibitory activity, 5f is moderate while other compounds 5a-d are weak against the (HepG2) cell lines, and have the following order: 5g > 5i >5e >5h> 5f > 5a > 5b > 5c > 5d. this indication of the substituents at the position phenyl group in arylhydrazo moiety affects the *in vitro* inhibitory activity. The electron-withdrawing introduction group (nitro group > bromine atom > chlorine atom) at the 4-position of the phenyl group. In contrast, the introduction of the electron-donating group (methoxy group > methyl group) reduces the anticancer activity

CONCLUSION

In summary, the new bis(arylazo)-terpyrazole derivatives 5a-i were synthesized *via* simple coupling reaction of sodium 3,3'-(5-methyl-1-phenyl-1*H*-pyrazole-3,4-diyl) bis(3-oxoprop-1-en-1-olate) (2) with arenediazonium salt, followed by condensation of the products 3a-i with hydrazine hydrate. The newly synthesized compounds structures were confirmed by tools spectrum and also from the calculation of the pKa to determine the best tautomeric form. The *in-vitro* anti-bacterial potential of the final synthesized compounds 5a-i was determined to achieve their antimicrobial potential.

ACKNOWLEDGMENTS : This work was supported by the Tabuk University Foundation of Saudi Arabia (Grant no. S-1440-0191).

CONFLICT OF INTEREST : None

FINANCIAL SUPPORT : None

ETHICS STATEMENT : None

REFERENCES

1. Dai H, Ge S, Guo J, Chen S, Huang M, Yang J, et al. Development of novel bis-pyrazole derivatives as antitumor agents with potent apoptosis induction effects and DNA damage. Eur J Med Chem. 2018;143:1066-761.
2. Zhi Y, Wang Z, Yao C, Li B, Heng H, Cai J, et al. Design and synthesis of 4-(heterocyclic substituted amino)-1H-pyrazole-3-carboxamide derivatives and their potent activity against acute myeloid leukemia (AML). Int J Mol Sci. 2019;20(22):5739-53.

3. Halawa AH, Eskndrani AA, Elgammal WE, Hassan SM, Hassan AH, Ebrahim HY, et al. Rational design and synthesis of diverse pyrimidine molecules bearing sulfonamide moiety as novel ERK inhibitors. Int J Mol Sci. 2019;20(22):5592-618.
4. Carrión MD, Cara LP, Camacho EV, Tapias M, Escames G, Castroviejo DA, et al. Entrena A. Pyrazoles and pyrazolines as neural and inducible nitric oxide synthesis (nNOS and iNOS) potential inhibitors (III). Eur J Med Chem. 2000;43(11):2579-91.
5. Tumietto F, Giacomell L. Fenticonazole: an effective topical treatment for superficial mycoses as the first-step of antifungal stewardship program. Eur Rev Med Pharm Sci. 2017;21(11):2749-56.
6. Darwish ES, Mahmoud FF, Altalbawy FMA. Synthesis and Antimicrobial Evaluation of Some New Pyrazole, Fused Pyrazolo[1,5-a] pyrimidine and Pyrazolo[1,5-d]pyrimido[4,5-d][1,2,3]triazine Derivatives. Asian J Chem. 2012;24(7):2997-3002.
7. Gökhan-Kelekçi N, Koyunoğlu S, Yabanoğlu S, Yelekçi K, Özgen Ö, Uçar G, et al. New pyrazoline bearing 4(3H)-quinazolinone inhibitors of monoamine oxidase: Synthesis, biological evaluation, and structural determinants of MAO-A and MAO-B selectivity. Bioorg Med Chem. 2009;17(2):675-89.
8. Abid M, Bhat AR, Athar F, Azam A. Synthesis, spectral studies and antiamoebic activity of new 1-N-substituted thiocarbamoyl-3-phenyl-2-pyrazolines. Eur J Med Chem. 2009;44(1):417-25.
9. Desai NC, Joshi SB. Synthesis and antimicrobial activity of some hybrid 2-aryl-4-(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-2,3-dihydro-1H benzo[b][1,4]diazepine derivatives. Indian J Chem. 2020;59B:238-46.
10. Pawar GG, Bineesh P, Kumar PSR, Rangnekar DW, Kanetkar VR. The synthesis and application of 3-arylazo-4-phenylthieno-2,3-c-isothiazole and ethyl-3-arylazo-4-phenylthieno-2,3-c-isothiazole-5-carboxylate. J Serb Chem Soc. 2005;70(6):799-805.
11. Altalbawy FMA. Synthesis and antimicrobial evaluation of some novel bis-α,β-unsaturated ketones, nicotinonitrile, 1,2-dihydropyridine-3-carbonitrile, fused thieno[2,3-b]pyridine and pyrazolo[3,4-b]-pyridine derivatives. Int J Mol Sci. 2013;14(2):2967-79.
12. Waring DR, Hallas G. The Chemistry and Application of Dyes.Plenum Press: New York, NY, USA; 1990. p. 381.
13. Matsuoka M. Infrared Absorbing Dyes. Plenum Press: New York, NY, USA; 1990. p. 95.
14. Garg HG, Prakash C. Potential antidiabetics. N1-(beta-hydroxybenzylmethyl)-3-methyl-4-arylhydrazono-2-pyrazolin-5-ones and N1-(beta-hydroxybenzylmethyl)-3-methyl-4-arylazo-5-methyl- or –phenylpyrazoles. J Med Chem. 1971;14(2):175-6.
15. Habibi MH, Hassanzadeh A, Zeini-Isfahani A. Spectroscopic studies of solophenyl red 3BL polyazo dye tautomerism in different solvents using UV-visible,1H NMR and steady-state fluorescence techniques. Dyes Pigm. 2006;69(1-2):93-101.
16. Otutu JO, Osabohien E, Efurhievwe EM. Synthesis and spectral properties of hetarylmonoazo dyes derived from 2-amino-5-nitrothiazole. Orient J Chem. 2011;27(4):1389-96.
17. Medhat M, El-Zaiat S, Altalbawy FMA, Saleh H. Characterization of the Physical Properties of Azo Merocyanine Dyes in Different Solvents and Concentrations. Optik. 2017;149:104-12.
18. Abdelhamid AO, Gomha SM, El-Enany WAMA. Convenient and efficient method for synthesis of bis-hetaryl ketones and evaluation of their antimicrobial activity. J Heterocycl Chem. 2019;56(2):426-33.
19. Abdelhamid AO, Gomha SM, El-Enany WAMA. Efficient synthesis and antimicrobial evaluation of new azolopyrimidines-bearing pyrazole moiety. J Heterocycl Chem. 2019;56(9):2487-93.
20. Gomha SM, Edrees MM, Altalbawy FMA. Design, Synthesis and characterization of some new bis-pyrazolyl-thiazoles incorporating the thiophene moiety as potent anti-tumor agents. Int J Mol Sci. 2016;17(9):1499-510.
21. Altalbawy FMA. Synthesis, in vitro antimicrobial, anticancer evaluation of some new pyridazines and polyfunctionally substituted heterocyclic compounds. Asian J Chem. 2015;27(12):4361-8.
22. Kheder NA, Altalbawy FMA. Synthesis, in vitro antimicrobial, anticancer evaluation of some novel bis-cyanoacrylamide and bis-azoles derivatives. Int J Pharm Pharm Sci. 2016;8:420-7.
23. Elgemeie G, Altalbawy F, Alfaidi M, Rania Azab R, Hassan A. Synthesis, characterization and antimicrobial evaluation of novel 5-benzoyl-N-substituted amino- and 5-benzoyl-N-sulfonylamino-4-alkylsulfanyl-2-pyridones. Drug Des Devel Ther. 2017;11:3389-99.
24. Altalbawy FMA, Darwish ESS. Synthesis and antimicrobial activity of 1,2,4-triazolo[4,3-b][1,2,4,5]-tetrazines. Asian J Chem. 2011;23(7):2951-5.

25. Darwish ES, Mahmoud FF, Altalbawy FA. Synthesis and antimicrobial evaluation of some New pyrazole, fused pyrazolo[1,5-a]pyrimidine and pyrazolo[1,5-d]pyrimido[4,5-d][1,2,3]-triazine derivatives. Asian J Chem. 2012;24(7):2997-3002.
26. Shawali AS, Mosselhi AM, Altalbawy FMA, Farghaly TA, Tawfik NM. Synthesis and tautomeric structure of 3,7-bis(arylazo)-6-methyl-2-phenyl-1H-imidazo-[1,2-b]pyrazoles in ground and excited states. Tetrahedron. 2008;64(23):5524-30.
27. Shawali AS, Abdelkader MH, Altalbawy FMA. Synthesis and tautomeric structure of novel 3,7-bis(arylazo)-2,6-diphenyl-1H-imidazo-[1,2-b]pyrazoles in ground and excited states. Tetrahedron. 2002;58(14):2875-80.
28. Altalbawy FMA, Darwish ESS, Abdelkader MH, Elnagdi MH. Synthesis, electronic absorption, fluorescence and life time spectroscopic study of some new coumarin dyes. Asian J Chem. 2016;28(10):2303-10.
29. Altalbawy FMA, Al-Sherbini EAM. Absorption, fluorescence, photochemical and thermal cis/trans isomerization reactivity of 1-methyl-4-(4′-aminostyryl)pyridinium iodide. Chem Sci Trans. 2015;4:1018-30.
30. Altalbawy FMA, Al-Sherbini EAM. Spectrophotometric determination of acidity constant of 1-methyl-4-[4'-aminostyryl]quinolinium iodide in aqueous buffer and micellar solutions in the ground and excited states. Asian J Chem. 2013;25:6181-5.
31. Shawali AS, Haboub AJM. Bis-enaminones as precursors for synthesis of novel 3,4-bis(heteroaryl)-pyrazoles and 3,6-bis-(heteroaryl)-pyrazolo[3,4-d]pyridazines. J Chem Res. 2011;35(6):341-5.
32. Shawali AS, Zeid IF, Abdelkader MH, Elsherbini AA, Altlbawy FMA. Synthesis, acidity constants and tautomeric structure of 7-arylhydrazono[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines in ground and excited states. J Chin Chem Soc. 2001;48(1):65-72.
33. Darwish ES, Mosselhi MA, Altalbawy FM, Saad HA. Synthesis, acidity constants and tautomeric structure of the diazonium coupling products of 2-(benzylsulfanyl)-7H-purin-6-one in its ground and excited. Molecules. 2011;16(10):8788-802.
34. Esmail R, Kurzer F. Heterocyclic compounds from urea derivatives. Part XXIII. thiobenzoylated thiocarbonohydrazides and their cyclization. J Chem Soc. 1975;18:1787-91.
35. Muanz DN, Kim BW, Euler KL, Williams L. Antibacterial and antifungal activities of nine medicinal plants from Zaire. Int J Pharmacogn. 1994;32(4):337-45.
36. Harborne JB, Williams CAA. survey of antifungal compounds from higher plants, 1982-1993. Phytochemistry. 1994;37(1):19-42.
37. Benzineb E, Kambouche N, Hamiani A, Bellahouel S, Zitouni H, Toumi H. Phenolics Compounds and Biological Activity of Leaves of Anabasis Articulata, an Algerian Medicinal Plant. Int J Pharm Res Allied Sci. 2019;8(4):1-5.
38. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.
39. Srivastava SK, Jha A, Agarwal SK, Mukherjee R, Burman AC. Synthesis and structure-activity relationships of potent antitumor active quinoline and naphthyridine derivatives. Anti-cancer Agents Med Chem. 2007;7(6):685-709.
40. Azadpour M, Farajollahi MM, Varzi AM, Hadipour F, Barati M. The evaluation of cytotoxicity effects of Rheum ribes L. (rubarb) extract on cancer cell lines and its antibacterial and mutagenicity activity. Entomol Appl Sci Lett. 2020;7(3):7-12.
41. Ahmad MS, Shawky A, Ghobashy M, Othman F, Ahmed RH. Effect of Some medicinal plants on life cycle of Citrus Brown Mites (Eutetranychus orientalis). Int J Pharm Res. Allied Sci. 2018;7(4):13-7.
42. Al-Awadi NA, Elnagdi MH, Ibrahim YA, Kaul K, Kumar A. Efficient synthesis of 3-aroylcinnolines from aryl methyl ketones. Tetrahedron. 2001;57(8):1609-14.
43. Shawali AS, Haboub AJM. Reaction of hydrazonoyl halides with bis-enaminones: A convenient route for synthesis of novel polyaza-terheterocycles. J Heterocycl Chem. 2013;50(1):17-22.
44. Altalbawy FMA, Darwish ESS. Synthesis and tautomeric structure of 7-arylhydrazono-3,5-diphenyl-5H-pyrazolo[5,1-c][1,2,4]triazol-6(7H)-ones in its ground and excited states. Asian J Spectrosc. 2012;16:45-54.
45. John CD. The Hammett Equation; Press Syndicate of Cambridge University: New York, NK, USA; 1973.

46. Altalbawy FMA, Al-Sherbini EAM. Spectrophotometric determination of acidity constant of 1-methyl-4-[4'-aminostyryl]quinolinium iodide in aqueous buffer and micellar solutions in the ground and excited states. Asian J Chem. 2013;25:6181-5.
47. Altalbawy F, Darwish E, Medhat M, El-Zaiat S, Saleh H. Synthesis, characterization, tautomeric structure and solvatochromic behavior of novel 4-(5-arylazo-2-hydroxystyryl)-1-methylpyridinium iodide as potential molecular photoprobe. Materials. 2016;9(12):1022-37.
48. Shawali AS, Darwish ESS, Altalbawy FMA. Synthesis of (4-Amino-5-phenyl-1,2,4-triazol-3-yl) thiohydrazonates and spectrophotometric study of their cyclization products in ground and excited states. Asian J Spectrosc. 2007;11:115-25.
49. Shier WT. Mammalian cell culture on $5 a day: a laboratory manual of low cost methods. Los Banos, University of the Philippines. 1991;64(8):9-16.