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Synthesis, Molecular Modeling and Biological Evaluation of Indeno[1,2-b]quinoxaline Derivatives as Antifungal and Antibacterial Agents

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THE (11H-indeno[1,2-b]quinoxalin-11-ylidene)thiocarbohydrazone were synthesized and consequently some new novel structures were obtained in a good yields. Their chemical structures were assigned by means of spectral analysis. Molecular modeling at B3LYP/6-31 g (d, p) is utilized to calculate both the optimized structure and vibrational spectra of some studied structures. A promising antimicrobial result was obtained from the new synthesized compounds.

Keywords: 11*H*-Indeno [1,2-*b*]quinoxalin-2-one, Thiocarbohydrazides, 2-Chloroacetamide derivatives, Molecular modeling, Biologically activity.

Introduction

11*H*-Indeno[1,2-*b*]quinoxalin-2-one is widely used for preparation of derivatives having a wide range of biological activity. Schiff bases obtained from the 11*H*-indeno[1,2-*b*]quinoxalin-2-one are highly cytotoxic and possess antiviral activity [1]. Quinoxalines are a versatile lead molecule for the design of potential bioactive agents and its derivatives were reported to possess broad spectrum of pharmacological activities such as anti-HIV [2, 3], anti-inflammatory [4, 5] anti-cancer [6-8] and activity as kinas inhibitor [9]. The indenoquinoxaline derivatives have significant applications in dyes and semiconductors [10-12]. Thiocarbohydrazides have gained increased interest in both synthetic organic chemistry and biological fields.

Contrary to the above, we attempted to synthesize the new 11*H*-indeno[1,2-*b*]quinoxalin-2-one derivatives which have good therapeutic potential of antimicrobial drugs. In addition, applying the theoretical studies on some synthesized compounds because of molecular modeling at different levels is important tool whereas experimental techniques are limited and/or unavailable. It could provide physical, chemical and biological data for many systems and molecules [13-17]

Experimental

Chemistry

All melting points were determined using an Electrothermal 9100 digital melting point apparatus. IR spectra were recorded on a Beckman infrared spectrophotometer PU 7712 (Beckman Instruments, USA) using KBr. ^{1}H and $^{13}CNMR$ spectra were recorded on Jeol JMS-AX 500 MHz using TMS as an internal standard, chemical shifts are expressed as δ (ppm). Mass spectra were recorded on Varian MAT 311 A at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Cairo University. Precoated silica gel 60 F254 plates with a layer thickness 0.25 nm from Merck were used for thin layer chromatography. Yields are not optimized.

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2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) thiocarbazone (3)

A mixture of 11*H*-indeno[1,2-*b*]quinoxalin-2-one **(1)** (0.5 mmol), mp 225–227°C [18] (mp 227–229 °C) [19] and thiocarbohydrazide **(2)** (0.5 mmol) [20] in ethanol absolute was heated under reflux for about 6 h. After the completion of the reaction (monitored by TLC petroleum ether/ ethyl acetate 3:1) the orange red precipitate was filtered off and recrystallized by ethanol. Yield: 80%; m.p. 273-275 °C. Anal. calcd for $C_{16}H_{12}N_6S$ (320) C, 59.98; H, 3.78; N, 26.23; S, 10.01; found , 59.85; H, 3.69; N, 26.13; S, 9.95%. IR (KBr): ν = 3443 (NH, NH_2), 1635 (C=C), 1505, 1358 (C=S) cm^{-1}. ^1H NMR (500 MHz, DMSO, d6): δ = 5.25 (br, 2H, NH_2, exchangeable with D_2O), 7.64-8.21 (m, 8 ArH), 10.86, 12.59 (2s, 2H, 2NH, exchangeable with D_2O), ppm. ^{13}C NMR (125 MHz, DMSO, d6): δ = 110.6, 117.1, 118.3, 126.3, 139.3, 140.9 (C=C), 141.9, 142.5, 153.9(C=N), 165.3 (C=S) ppm. MS (70 eV): *m*/*z* (%) = 320 (20) $[M]^+$.

General procedure for the synthesis of compounds 6a-f and 7a-f

A solution of the appropriate 2-chloro-N-arylacetamide **5** (10 mmol) (which were prepared according to the reported method [21] in 20 ml acetone was added to a solution of 3.52 g 2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) thiocarbazone **(3)** (10 mmol) in 20 ml acetone (**4a**) containing 1.38 g K_2CO_3 (10 m mol). The reaction mixture was refluxed for 4 h, and then the reaction mixture was poured onto ice water and washed 4 times with water. The solid precipitate was formed, collected, dried, and recrystallized to give compounds **6a-f**. When the same reactions were carried out in 2-butanone (**4b**) led to the formation of **7a-f**.

2-(1-(2-(11H-indeno[1,2-b]quinoxalin-11-ylidene)hydrazinecarbonothioyl)-2-(propan-2-ylidene)hydrazinyl)-N-(4-methoxyphenyl) acetamide (6a)

Brownish red powder, yield: 73%; m.p. 229-231°C. Anal. calcd for $C_{28}H_{25}N_7O_2S$(523): C, 64.23; H, 4.81; N, 18.73; S, 6.12; found C, 64.17; H, 4.73; N, 18.62; S, 6.08%.; IR (KBr): *ν* = 3444(NH), 1646 (CO), 1543(C=C), 1391 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 2.04, 2.24 (2s, 2 CH_3 group), 3.72 (s, 3 H, OMe) 3.97 (s, 2H, CH_2), 6.87-8.11 (m,12H, 12 ArH), 10.07, 13.56 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS (70 eV): *m*/*z* (%) = 523 (5) $[M]^+$.

2-(1-(2-(11H-indeno[1,2-b]quinoxalin-11-ylidene)hydrazinecarbonothioyl)-2-(propan-2-ylidene)hydrazinyl)-N-phenylacetamide (6b)

Brownish red powder, yield: 68%; m.p. 251-253°C. Anal. calcd for $C_{27}H_{23}N_7OS$(493): C, 65.70; H, 4.70; N, 19.86; S, 6.50; found C 65.62, H 4.62, N 19.79, S, 6.45%.; IR (KBr): *ν* = 3437 (NH), 1634 (C=O), 1555 (C=N), 1378 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 2.03, 2.22 (2s, 2 CH_3 group), 4.00 (s, 2H, CH_2), 7.05-8.16 (m, 13 ArH), 10.20, 13.52 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. ^{13}C NMR (125 MHz, $DMSOd_6$): δ = 18.4, 26.0 (2 CH_3), 34.2 (CH_2), 119.8, 122.5, 124.1, 129.0, 129.7, 129.9, 130.6, 130.9, 132.1(C=C), 140.6, 153.7, 158.9(C=N), 164.4(C=O), 167.8(C=S) ppm. MS (70 eV): *m*/*z* (%) = 493 (8) [M+].

2-(1-(2-(11H-indeno[1,2-b]quinoxalin-11-ylidene)hydrazinecarbonothioyl)-2-(propan-2-ylidene)hydrazinyl)-N-(4-fluorophenyl)acetamide (6c)

Brownish red powder, yield: 75%; m.p. 254-256°C. Anal. calcd for $C_{27}H_{22}FN_7OS$(511): C, 63.39; H, 4.33; F, 3.71; N, 19.17; S, 6.27; found C, 63.31; H, 4.25; F, 3.65; N, 19.05; S, 6.18%.; IR (KBr): *ν* = 3436(NH), 1645(CO), 1629, 1368 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 2.03, 2.22 (2s, 2 CH_3 group), 4.00 (s, 2H, CH_2), 7.05-8.16 (m,12H, 12 ArH), 10.20, 13.52 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS (70 eV): *m*/*z* (%) = 511 (14) $[M]^+$.

2-(1-(2-(11H-indeno[1,2-b]quinoxalin-11-ylidene)hydrazinecarbonothioyl)-2-(propan-2-ylidene)hydrazinyl)-N-(3,5-dichlorophenyl) acetamide (6d)

Brownish red powder, yield: 67%; m.p. 242-244°C. Anal. calcd for $C_{27}H_{21}Cl_2N_7OS$(561): C, 57.65; H, 3.76; Cl, 12.61; N, 17.43; S, 5.70; found C, 57.56; H, 3.63; Cl, 12.55; N, 17.34; S, 5.75%.; IR (KBr): *ν* = 3432(NH), 1632(CO), 1563(C=C), 1388 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 2.08, 2.25 (2s, 2 CH_3 group), 4.10 (s, 2H, CH_2), 7.42-8.17 (m, 11 ArH), 9.27, 9.89 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS (70 eV): *m*/*z* (%) = 565 (70) $[M^+ +3]$.

2-(1-(2-(11H-indeno[1,2-b]quinoxalin-11-ylidene)hydrazinecarbonothioyl)-2-(propan-2-ylidene)hydrazinyl)-N-(4-(N-(pyridin-2-yl) sulfamoyl)phenyl)acetamide (6e)

Brownish red powder, yield: 77%; m.p. 313-351°C. Anal. calcd for $C_{32}H_{27}N_9O_3S_2$ (649): C, 59.15; H, 4.19; N, 19.40; S, 9.87; found C, 59.08; H, 4.11; N, 19.32; S, 9.79%.; IR (KBr): *ν*

= 3437(NH), 1632(CO), 1508(C=C), 1376 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 2.08, 2.20 (2s, 2 CH_3 group), 4.80 (s, 2H, CH_2), 7.16-8.21 (m, 16 ArH), 9.85, 10.26, 10.60 (3 s, 3H, 3 NH, exchangeable with D_2O) ppm. MS (70 eV): *m/z* (%) = 649 (15) $[M]^+$.

2-(1-(2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) hydrazinecarbonothioyl)-2-(propan-2-ylidene) hydrazinyl)-N-(thiazol-2-yl)acetamide (6f)

Brownish red powder, yield: 69%; m.p 243-245°C. Anal. calcd for $C_{24}H_{20}N_8OS_2$ (500): C, 57.58; H, 4.03; N, 22.38; S, 12.81; found C, 57.51; H, 3.99; N, 22.29; S, 12.74%.; IR (KBr): *v* = 3433(NH), 1632(CO), 1550(C=N), 1382 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.98, 2.38 (2s, 2 CH_3 group), 4.22 (s, 2H, CH_2), 6.53, 6.92 (2 d, J_{HH} = 4.0 Hz, 2 H, thiazole protons), 6.83-8.65 (m, 10H, 2 NH, exchangeable with D_2O, 8 ArH) ppm. ^{13}C NMR (125 MHz, $DMSOd_6$): δ = 18.2, 26.9 (2 CH_3), 33.5 (CH_2), 107.0, 113.9, 121.1, 122.7, 129.0, 129.8, 130.5, 131.4, 132.6, 135.0, 137.0, 138.2(C=C), 140.1, 141.5, 147.2, 156.3, 158.6, 161.6(C=N), 164.4(CO), 169.3, 183.9 (C=S)ppm. MS (70 eV): *m/z* (%) = 500 (5) $[M]^+$.

2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) hydrazinecarbonothioyl)-2-(butan-2-ylidene) hydrazinyl)-N-(4-methoxyphenyl)acetamide (7a)

Brownish red powder, yield: 67%; m.p. >350°C. Anal. calcd for $C_{29}H_{27}N_7O_2S$ (537): C, 64.79; H, 5.06; N, 18.24; S, 5.96; found C, 64.71; H, 4.98; N, 18.17; S, 5.89%.; IR (KBr): *v* = 3436(NH), 2926(CH), 1627(CO), 1505(C=C), 1377 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.28 (t, J_{HH} = 8 Hz, 3H, CH_2-**CH_3** group), 2.02 (s, 3 H, CH_3), 2.44 (q, J_{HH} = 8 Hz, 2 H, -**CH_2**-CH_3) 3.73 (s, 3H, OCH_3), 3.96 (s, 2 H, CH_2), 6.88-8.16 (m, 12 ArH), 10.09, 13.28 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS (70 eV): *m/z* (%) = 537 (10) $[M]^+$.

2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) hydrazinecarbonothioyl)-2-(butan-2-ylidene) hydrazinyl)-N-phenylacetamide (7b)

Brownish red powder, yield: 73%; m.p 251-253°C. Anal. calcd for $C_{28}H_{25}N_7OS$ (507): C, 66.25; H, 4.96; N, 19.32; S, 6.32; found C, 66.18; H, 4.88; N, 19.25; S, 6.27%.; IR (KBr): *v* = 3437 (NH), 1652 (C=C), 1561 (C=N), 1381 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.27 (t, J_{HH} = 8 Hz, 3H, CH_2-**CH_3** group), 2.01 (s, 3 H, CH_3), 2.19 (q, J_{HH} = 8 Hz, 2 H, -**CH_2**-CH_3) 4.00 (s, 2 H, CH_2), 7.04-8.13 (m, 13H, 13 ArH), 10.25, 13.24 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS (70 eV): *m/z* (%) = 507 (4) $[M]^+$.

2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) hydrazinecarbonothioyl)-2-(butan-2-ylidene)-hydrazinyl)-N-(4-fluorophenyl)acetamide (7c)

Brownish red powder, yield: 80%; m.p. 239-241°C. Anal. calcd for $C_{28}H_{24}FN_7OS$ (525): C, 63.98; H, 4.60; F, 3.61; N, 18.65; S, 6.10; found C, 63.89; H, 4.61; F, 3.55; N, 18.58; S, 6.03%.; IR (KBr): *v* = 3434 (NH), 1631 (C=C), 1369 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.29 (t, J_{HH} = 8 Hz, 3H, CH_2-**CH_3** group), 2.00 (s, 3 H, CH_3), 2.46 (q, J_{HH} = 8 Hz, 2 H, -**CH_2**-CH_3) 3.97 (s, 2 H, CH_2), 7.14-8.01 (m, 12H, 12 ArH), 10.28, 13.11 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS (70 eV): *m/z* (%) = 525 (6) $[M]^+$.

2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) hydrazinecarbonothioyl)-2-(butan-2-ylidene) hydrazinyl)-N-(3,5-dichlorophenyl)acetamide (7d)

Brownish red powder, yield: 83%; m.p. 243-245°C. Anal. calcd for $C_{28}H_{23}Cl_2N_7OS$ (575): C, 58.33; H, 4.02; Cl, 12.30; N, 17.01; S, 5.56; found C, 58.26; H, 3.92; Cl, 12.19; N, 16.95; S, 5.48%.; IR (KBr): *v* = 3438, 1635, 1373 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.30 (t, J_{HH} = 8 Hz, 3H, CH_2-**CH_3** group), 2.06 (s, 3 H, CH_3), 2.34 (q, J_{HH} = 8 Hz, 2 H, -**CH_2**-CH_3) 4.08 (s, 2 H, CH_2), 7.42-8.18 (m, 11H, 11 ArH), 9.85, 13.34 (2s, 2 H, 2NH, exchangeable with D_2O) ppm. MS (70 eV): *m/z* (%) = 575 (5) $[M]^+$.

2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) hydrazinecarbonothioyl)-2-(butan-2-ylidene) hydrazinyl)-N-(4-(N-(pyridin-2-yl)sulfamoyl) phenyl)acetamide (7e)

Brownish red powder, yield: 73%; m.p. 278-280°C. Anal. calcd for $C_{33}H_{29}N_9O_3S_2$ (663): C, 59.71; H, 4.40; N, 18.99; S, 9.66 found C, 59.63; H, 4.34; N, 18.78; S, 9.57%.; IR (KBr): *v* = 3441(NH), 1632(CO), 1506(C=C), 1365 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.24 (t, J_{HH} = 8 Hz, 3H, CH_2-**CH_3** group), 1.91 (s, 3 H, CH_3), 2.44 (q, J_{HH} = 8 Hz, 2 H, -**CH_2**-CH_3) 4.01 (s, 2 H, CH_2), 6.88-8.16 (m,12H, 12 ArH), 10.57, 12.45, 13.14 (3s, 3H, 3 NH, exchangeable with D_2O) ppm. MS (70 eV): *m/z* (%) = 663 (6) $[M]^+$.

2-(11H-indeno[1,2-b]quinoxalin-11-ylidene) hydrazinecarbonothioyl)-2-(butan-2-ylidene) hydrazinyl)-N-(thiazol-2-yl)acetamide (7f)

Brownish red powder, yield: 77%; m.p. 235-237°C. Anal. calcd for $C_{25}H_{22}N_8OS_2$(514): C, 58.35; H, 4.31; N, 21.77; S, 12.46 found C, 58.27; H, 4.22; N, 21.65; S, 12.41%.; IR (KBr): *v* = 3441(NH), 1634(CO), 1379 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.26 (t, J_{HH} = 7

Hz, 3H, CH_2-**CH_3** group), 1.91 (s, 3 H, CH_3), 2.18 (q, J_{HH} = 7 Hz, 2 H, -**CH_2**-CH_3) 4.11 (s, 2 H, CH_2), 6.54-8.18 (m,10H, 10 ArH), 12.39, 13.25, 13.62 (3s, 3H, 3 NH, exchangeable with D_2O) ppm. MS (70 eV): *m*/*z* (%) = 514 (3) $[M]^+$.

General procedure for the synthesis of compounds 8

To the mixture of **3** and ketones **4**, the potassium carbonate was added then refluxed for about 3h, and the in organic material filtered off and the solvent evaporated. The solid precipitate was formed, collected, dried, and recrystallized from methanol to give compounds **8**.

1-[(11H-indeno[1,2-b]quinoxalin-11-ylidene) amino]-3-[(propan-2-ylidene)amino] thiourea (8a)

Deep red powder, yield: 83%; m.p. 290-292°C. Anal. calcd for $C_{19}H_{16}N_6S$(360): C, 63.31; H, 4.47; N, 23.32; S, 8.90 found C, 63.25; H, 4.35; N, 23.22; S, 8.82%.; IR (KBr): ν = 3431(NH), 1629(CO), 1516(C=C), 1393 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.26, 2.17 (2s, 2 CH_3 group), 7.69-8.22 (m,8H, 8 ArH), 11.24, 14.01 (2s,2H, NH, exchangeable with D_2O) ppm. MS (70 eV): *m*/*z* (%) = 360 (5).

1-[(11H-indeno[1,2-b]quinoxalin-11-ylidene) amino]-3-[(butan-2-ylidene)amino] thiourea (8b)

Deep brown powder, yield: 83%; m.p 253-255°C. Anal. calcd for $C_{20}H_{18}N_6S$(374): C, 64.15; H, 4.85; N, 22.44; S, 8.56 found C, 64.08; H, 4.72; N, 22.33; S, 8.48%.; IR (KBr): ν = 3433(NH), 1622 (CO), 1501(C=C), 1383 (C=S) cm^{-1}; ^1H NMR (500 MHz, $DMSOd_6$): δ = 1.16 (t, J_{HH} = 7 Hz, 3H, CH_2-**CH_3** group), 2.01 (s, 3 H, CH_3), 2.14 (q, J_{HH} = 7 Hz, 2 H, -**CH_2**-CH_3), 6.44-8.08 (m, 8H, 8 ArH), 13.15, 13.43 (2s, 2H, 2 NH, exchangeable with D_2O) ppm. MS (70 eV): *m*/*z* (%) = 374 (25) $[M]^+$.

Theoretical Calculations details

Three model molecules were constructed as indicated in figure 1-a,b, c. All the studied model molecules were calculated using Gaussian 09 [22] soft code which implemented on workstation at spectroscopy department National Research Centre, Egypt. The structures were optimized with density functional theory method at B3LYP [23-25] level of theory using 6-31g (d, p). Vibrational spectra of the studied structure were calculated also at the same level of theory.

Biology

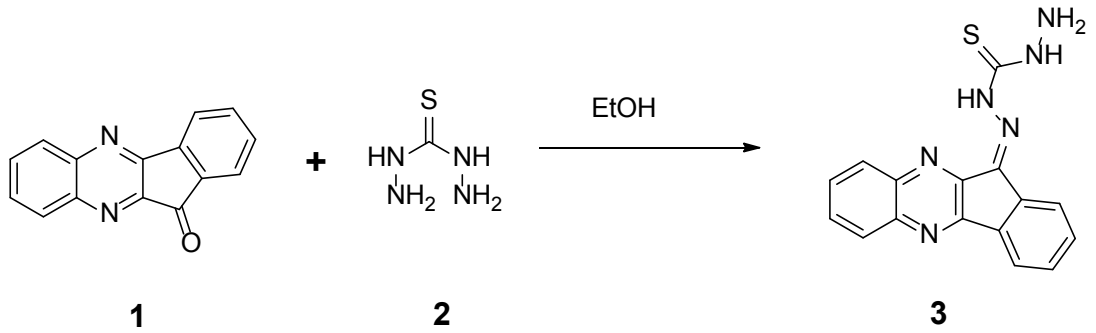
The antibacterial and antifungal activities were carried out in the Department of Microbial Chemistry, National Research Centre. The antimicrobial potential of chemical samples under study was tested using two Gram-positive bacteria (*Bacillus cereus,* and *Staphylococcus aureus* ATCC 6538), three Gram-negative bacteria (*Escherichia coli* NRRN 3008*, Pseudomonas aeruginosa* ATCC10145 and *Salmonella typhimurium* ATCC 25566) and one yeast, *Candida albicans*. Bacterial strains were cultured overnight at 37°C in Nutrient broth medium (5 g peptone, 3 g meat extract and 1000 ml distilled water) while yeast strain was cultured overnight at 37°C using potato dextrose medium. For antimicrobial test 15 gram of agar was added to the above-mentioned media to prepare Nutrient agar and potato dextrose agar plates.

The antimicrobial activity was determined by disc diffusion method as described by Vander and Vlientnck, 1991 [26]. Briefly, 100 µl of suspension of the tested microorganisms, containing 10^6 colony forming units (CFU)/ml of bacteria and 10^5CFU/ml of yeast were spread on nutrient agar and potato dextrose agar plates, respectively. The samples were suspended in DMSO. The filter paper discs (6 mm in diameter) was individually impregnated with diluted samples and leave them to evaporate the solvent then, placed on the agar plates which had previously been inoculated with the tested microorganisms. The disc with only solvent was used as a negative control. Plates were incubated at 37°C for 24h. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones and comparing with the control reference antibiotics streptomycin sulphate.

Results and Discussions

Chemistry

Boiling of equimolar amounts of indenoquinoxaline **1** with thiocarbohydrazide (**2)** in ethanol afforded (11H-indeno[1,2-b] quinoxalin-11-ylidene)thiocarbazone (**3)** in 80% yields. The structure of **3** was confirmed by IR, MS, ^1H and ^{13}C NMR spectra (Scheme 1). The IR spectrum of **3** contained a broad absorption bands at 3443 cm^{-1} due to stretching vibration of NH and NH_2 bonds. Absence of carbonyl (C=O) peak revealed the formation of **3**. In the ^1H NMR spectrum of **3**, we observed three signals at δ 5.25, 10.84 and 12.59 ppm due to NH_2 and 2 NH, respectively, as well as multiplets at about δ 7.64-8.21 ppm due to 8 Aromatic protons. In the ^{13}C NMR spectra of compound **3** the characteristic C=S group give rise to the signal at 165.3 ppm. Additional evidence supporting this structure was obtained by mass spectrum, which gave a molecular ion peak at *m/z* 320 (M^+)



1 **2** **3**

Scheme 1. Synthesis of(11H-indeno[1,2-b]quinoxalin-11-ylidene)thiocarbazone (3).

The condensation reaction between (11H-indeno[1,2-b]quinoxalin-11-ylidene)-thiocarbazone (**3**) and 2-chloro-N-acetamide derivatives **5** in acetone or 2-butanone **4a,b** in the presence of potassium carbonate (three component system) was investigated to give 2-(1-(2-(11H-indeno[1,2-b] quinoxalin-11-ylidene)hydrazinecarbonothioyl)-2-(propan-2-ylidene)- hydrazinyl)-N-(4-methoxyphenyl) acetamide (**6a)** or 2-(11H-indeno[1,2-b]quinoxalin-11-ylidene)hydrazine carbonothioyl)-2-(butan-2-ylidene) hydrazinyl)-N-(4-methoxy-phenyl)acetamide **(7a),** respectively (Scheme 2).

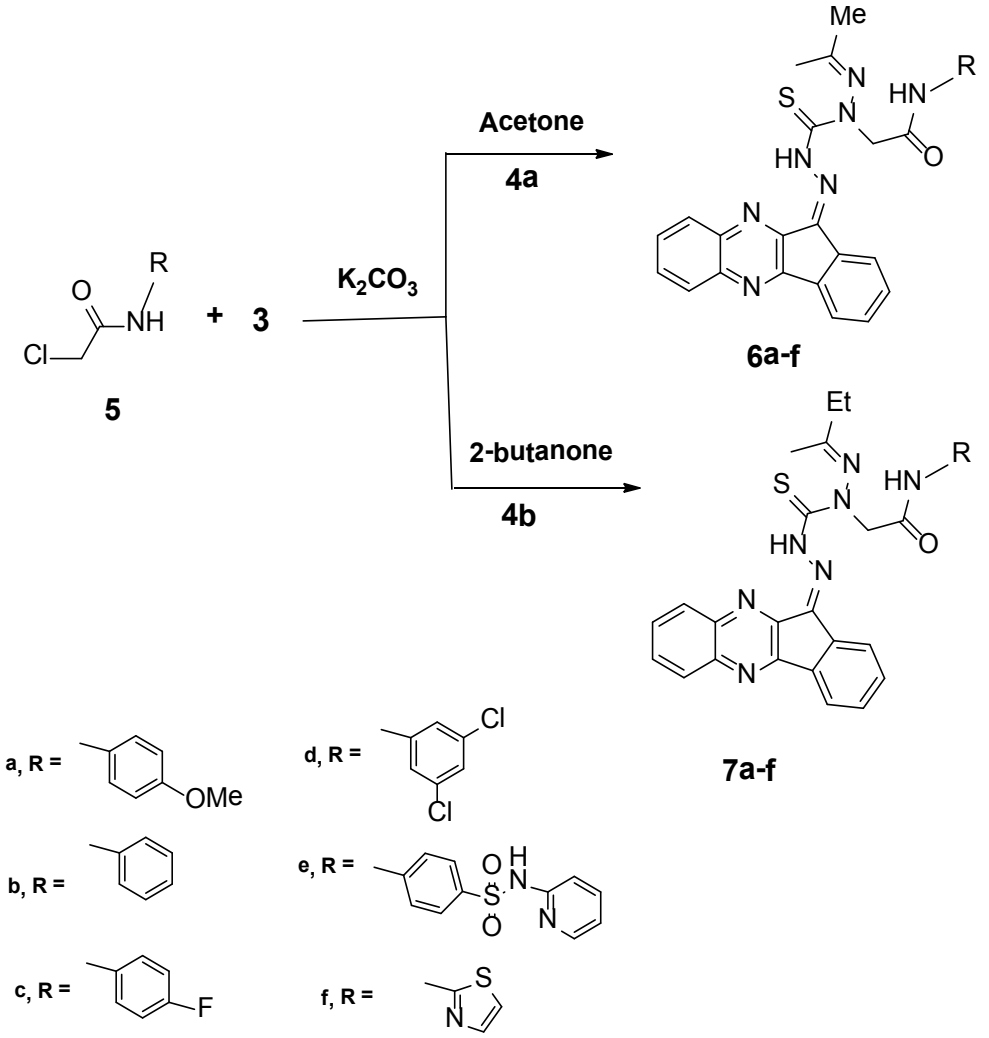
The structures of compounds **6a-f** and **7a-f** were deduced from their elemental analyses and their IR, ^{1}H, ^{13}C NMR and Mass spectroscopic measurements. Most compounds sparingly soluble in DMSO which is the main reason the ^{13}C NMR was not possible. Also, because we use high resolution 500 MHz for HNMR, we could conduct proton measurements in NMR apparatus. All compounds have shown an excellent agreement between calculated and experimentally obtained data for CHN elemental analysis. For example the ^{1}H NMR spectrum of **6a** exhibited four singlet signals at δ 2.04, 2.24, 3.72 and 3.97 ppm attributed to two methyl groups, methoxy group and CH_2 protons, respectively. The aromatic protons appeared as multiplets at δ = 6.88-8.11 along with the two singlet at 10.07 and 13.56 ppm exchangeable with D_2O related to two NH. On the other hand, ^{1}H NMR spectra of **7a** shows two signals for ethyl group at δ 1.28 (t, J_{HH} = 8 Hz, 3H, CH_2-**CH_3** group) and2.44 (q, J_{HH} = 8 Hz, 2 H,-**CH_2**-CH_3). The spectrum exhibited also five singlet at δ 2.02, 3.73, 3.96, 10.09 and 13.28 ppm attributed to CH_3, OCH_3, CH_2 and 2 NH The aromatic protons (12 ArH) appeared as a mutiplets at δ 6.88-8.16 ppm which in agreement with the proposed structure. **(c.f: experimental)**

Finally, reacting compound **3** with acetone **4a** or ethyl methyl ketone **4b** in the presence of potassium carbonate under reflux for 5hr. with TLC monitoring give 1-[(11H-indeno[1,2-b]quinoxalin-11-ylidene)amino]-3-[(propan-2-ylidene)amino] thiourea **(8a)** or 1-[(11H-indeno[1,2-b]quinoxalin-11-ylidene)amino]-3-[(butan-2-ylidene)amino] thiourea **(8b)** in 78 or 75 % yield respectively (Scheme 3). The condensation products **8** were investigated by elemental analyses, IR, ^{1}H NMR and Mass spectroscopic measurements. ^{1}H NMR spectra of **8a** showed the four singlet signals in the region δ 1.26, 2.17, 11.24 and 14.01 ppm, belonging to the protons of 2 CH_3 group and 2 NH. **(c.f: experimental)**

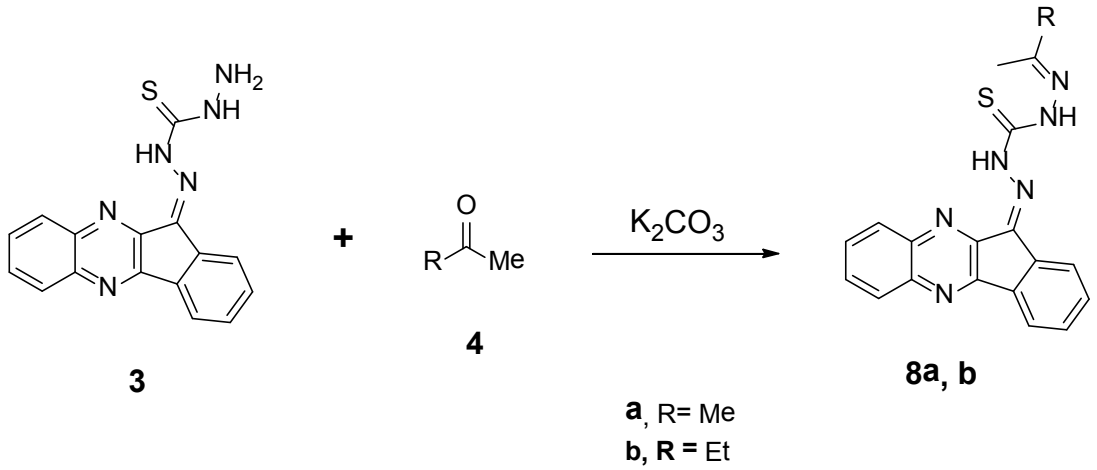
Molecular modeling

Three model molecules are representing the studied structures **3** and **6a** [keto-thione **A** and hydroxy-thione **B**] as shown in figure 1. They subjected to geometry optimization at DFT with B3LYP/6-31 G (d, p). The discrete Fourier transform (DFT) calculations indicated that the three structures are corresponding to the energy minimum which an indication that these structures are real and not corresponding to transition state and/or imaginary structures.

Total dipole moment (TDM) and HOMO/LUMO band gap energy (ΔE) were calculated for all model structures using DFT theory at B3LYP / 6-31 G (d, p) basis set (Figures 2-4). From HOMO/LUMO calculations TDM and HOMO/LUMO band gap energy ΔE were obtained as shown in table 1. For the first model structure TDM and HOMO/LUMO band gap energy ΔE were equal 4.8279 Debye and 3.0896 eV, respectively while for second and third model structure were 4.5036, 2.7603 Debye and 3.1171, 3.2847 eV. The increasing in TDM with ΔE decreasing is an indicator for stability and reactivity. Accordingly, TDM and HOMO/LUMO band gap energy ΔE result indicates that the probability of keto-thione **A** is more stable and more reactive than hydroxy-thione **B**.



Scheme 2. Synthesis of -(11H-indeno[1,2-b]quinoxalin-11-ylidene)hydrazinecarbonothioyl)-2-(propan or butan-2-ylidene)hydrazinyl)acetamide derivatives (6a-f) and (7a-f).



Scheme 3. Synthesis of thiourea derivatives 8.

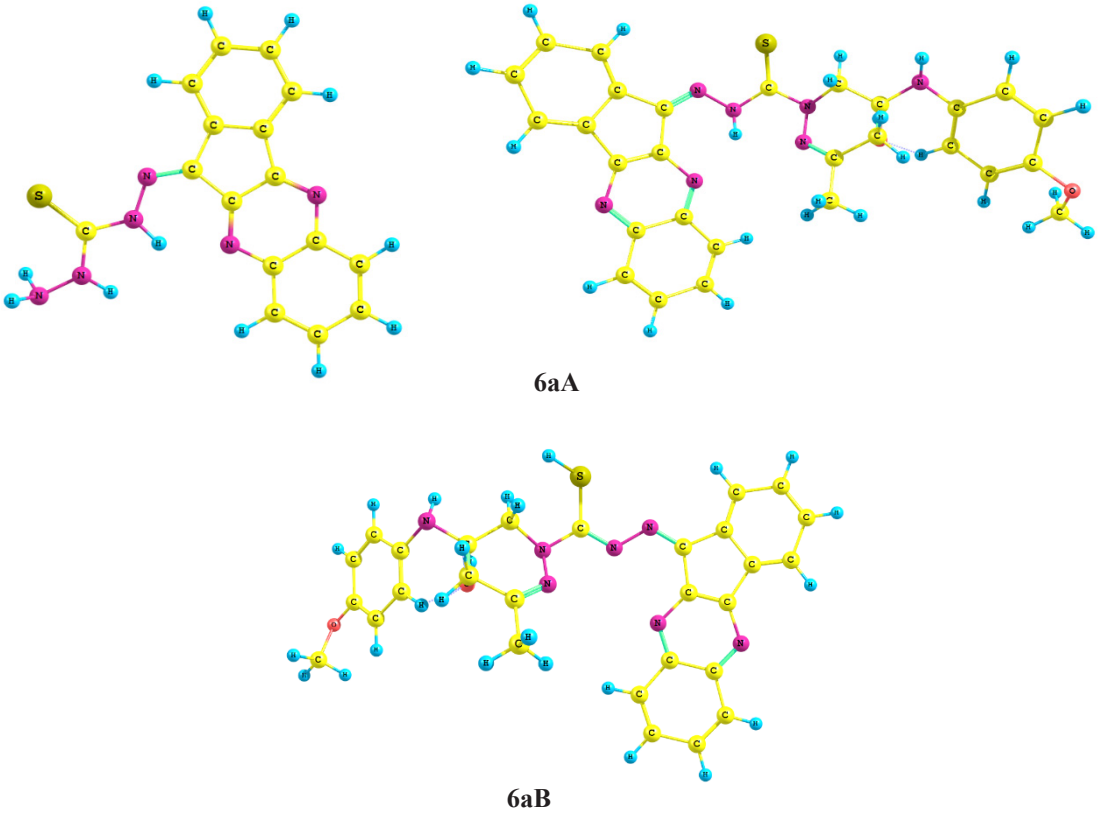


Fig. 1. Calculated B3LPY/6-31G (d, p) optimized structure for 3, 6aA and 6aB .

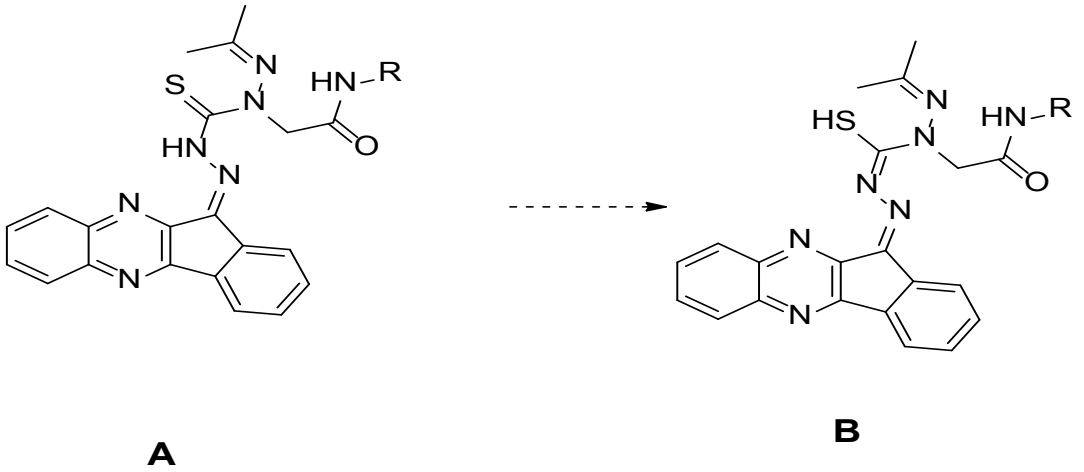


TABLE 1. Total dipole moment TDM (Debye) and HOMO/LUMO band gap energy ΔE (eV) Using B3LYP/ 6-31G (d, p) for 3, 6aA and 6aB

Structures	TDM	ΔE
3	4.8279	3.0896
6aA	4.5036	3.1171
6aB	2.7603	3.2847

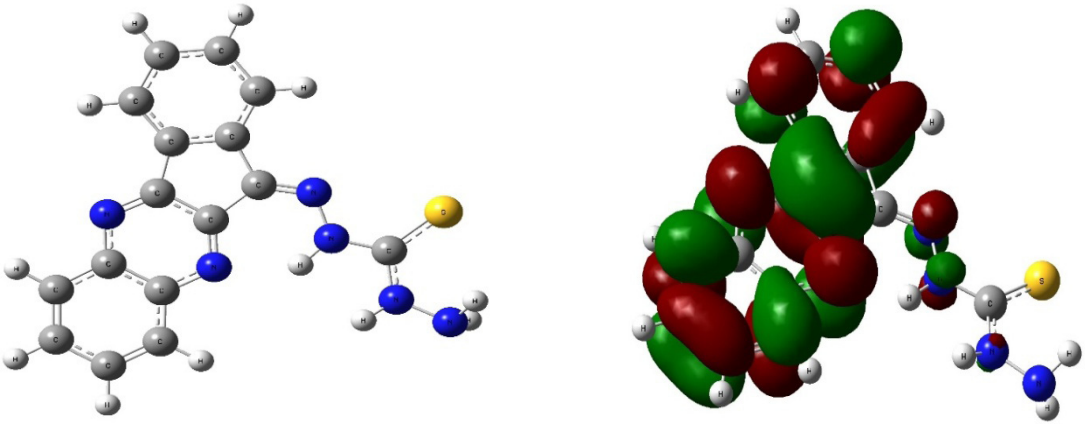


Fig.2. Calculated B3LPY/6-31g (d, p) HOMO/LUMO for 3.

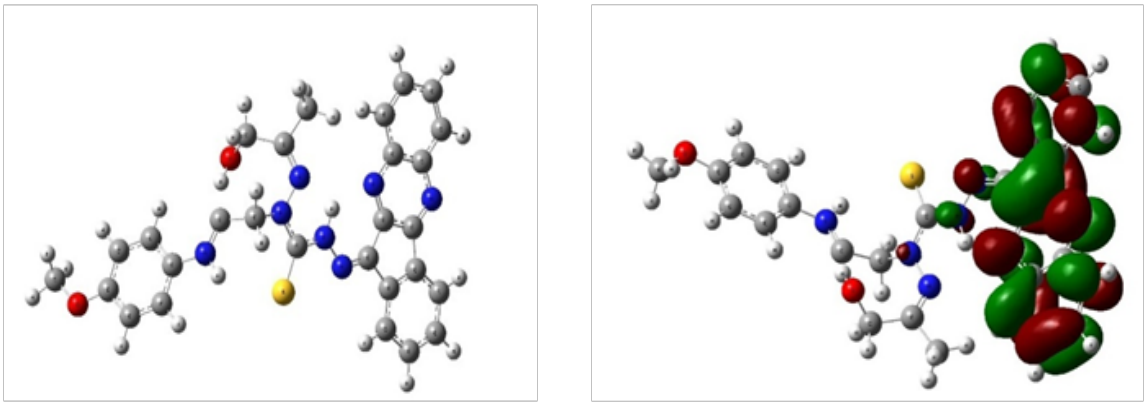


Fig. 3. Calculated B3LPY/6-31g (d, p) HOMO/LUMO for 6aA [keto-thione].

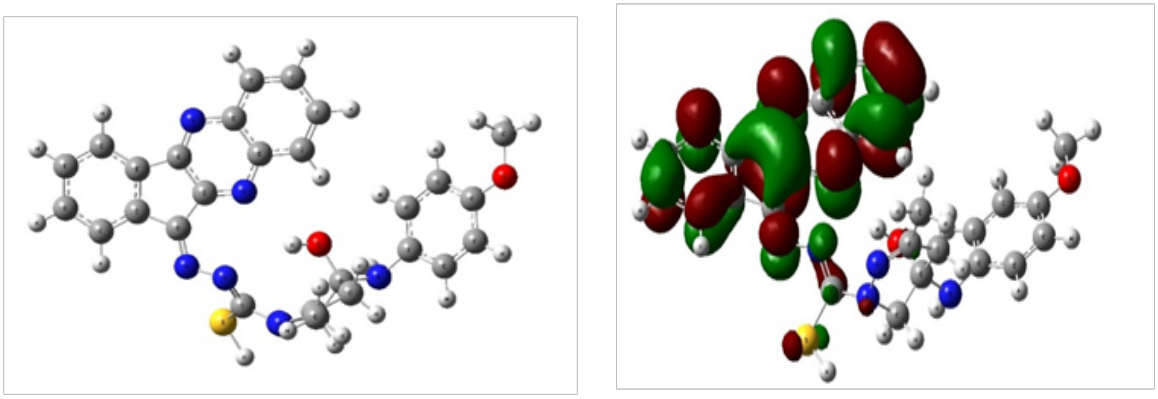
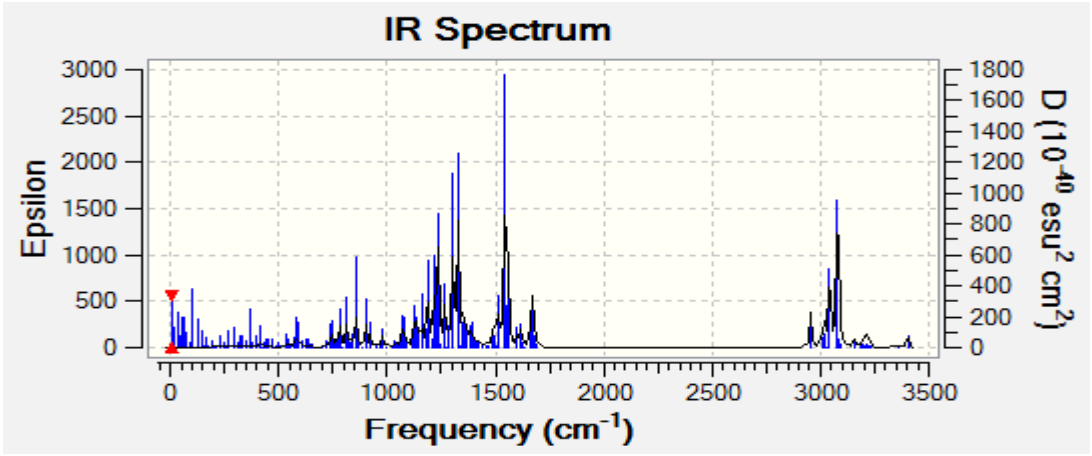
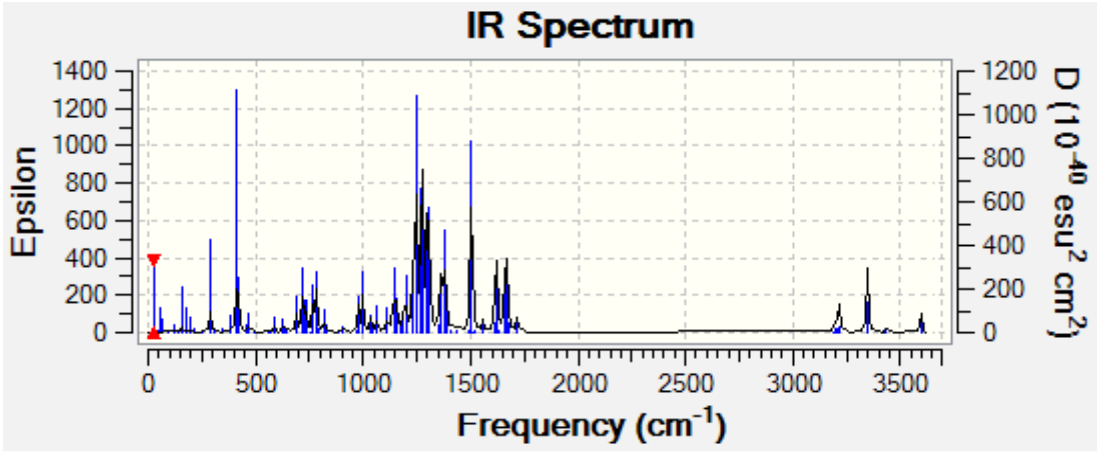


Fig.4. Calculated B3LPY/6-31g (d, p) HOMO/LUMO for 6aB [hydroxy-thione].

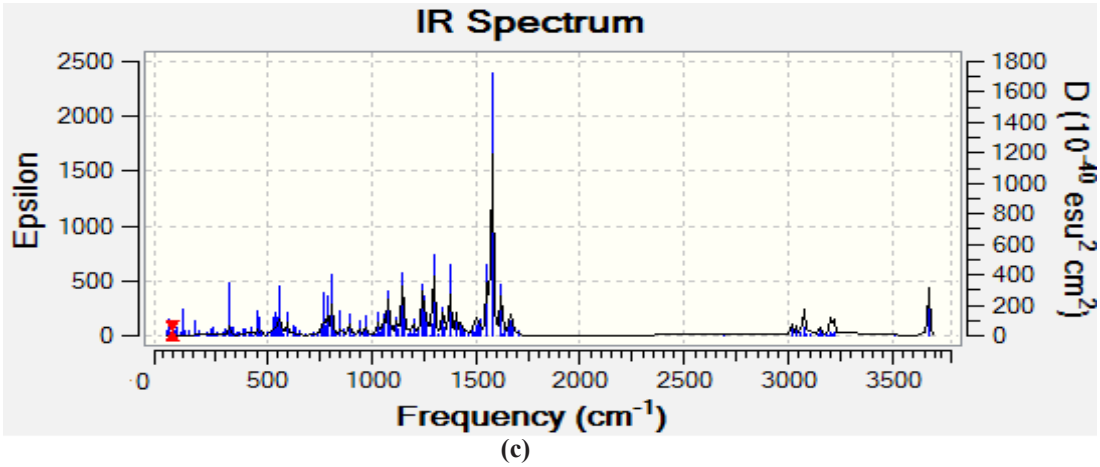
Another test for the structure could be achieved throughout calculated vibrational spectra. As indicated in figure 5-a, b and c. The obtained spectra are positive with no negative frequencies; this is another indication for the optimized structure is real structure. The assignment of the calculated spectra is not the point of discussion as it is calculated to confirm that the models are representing real structures.



(a)



(b)



(c)

Fig.5. Calculated B3LPY/6-31g (d, p) vibrational spectra for 3, 6aA and 6aB Biological Activity.

The antimicrobial screening of 7 new synthesized compounds (**1,3, 6c, 6f,7a,7d, 8b**) was carried out against different Gram positive (Bacillus cereus and staphylococcus aureus), Gram-negative (Escherichia coli and pseudomonas aeruginose) bacteria, as well as against the Candida Albicans fungi, compared to streptomycin sulphate as reference for antimicrobial (Table 2). From Table 2, compound **3** revealed broader spectrum of activity against all the tested organisms as compared to those of other compounds. Concerning Gram positive bacteria, compounds **1** and **3** were the most active as Bacillus Cereus inhibitors, showing 30% inhibition compared to the reference drug, streptomycin sulphate (0%). While in case of Staphylococcus Aureus strains they showed a remarkable inhibition, with 20% inhibition (for **1**) and 23% (for **3**), compared to the reference drug, streptomycin sulphate (20%). Regarding Gram-negative bacteria, compounds **1** and **3** demonstrated high activities among the tested samples, exhibiting inhibition (25% for **1** and 22% for **3**) against strains of Pseudomonas Aeruginosas, versus to 25% inhibition of streptomycin sulphate. Interestingly, compounds **3** and **8b** displayed a significant inhibition against Escherichia Coli strains with 20% (for **3**), which is the same as streptomycin sulphate (20%) and 12% (for **8b**). On the other hand, compounds **1** and **3** showed activity against the fungus, Candida Albicans with 30% inhibition and higher than the reference drug, streptomycin sulphate (22%). Based on the above mentioned data, it seems that the examined indenoquinoxaline (**1**) and (11H-indeno[1,2-b]quinoxalin-11-ylidene)-thiocarbazone (**3)** exhibit potent antimicrobial activity. In an old report [27] to investigation similar to this work, the biological effect was to the contrary this report very high. From our point view, the cause for the poor biological activity is because of the amino group lost its biological activity by losing its biological hydrogen atom. The bioactive compounds might have several invasive targets that could lead to inhibition of the microorganisms [28].

In table 3 we compare the antimicrobial activity for compound **1** and **3** (exhibit the good antimicrobial activity) with the reference drug (Streptomycin sulphate) at different concentration.

To define the effect of streptomycin sulphate antibiotic-samples (**1**, **3**, **8a** and **8b)** interaction on activity against 4 strains of various species of bacteria and strain of fungi as shown in Table 4, we found that, compounds **3**, **8a** and **8b** inhibited the growth of strains gram-negative bacteria *Pseudomonas aeruginosa and inhibited* the growth of strain of fungi *Candida albicans* with concentration mixture125-15 ug.

TABLE 2. Antimicrobial activitys of substances that showed poor or high activities. Substances with no antimicrobial activity were excluded.

Test sample at 420µg/ disc	**Inhibition zone (mm) of**				
	Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Bacillus cereus***	***Candida albicans***
Reference antibiotic Streptomycin sulphate	20	20	25	0	22
1	0	20	25	30	30
3	20	23	22	30	30
6c	0	6.5	0	0	0
6f	0	7	0	0	0
7a	7	0	0	0	0
7d	9	0	0	0	0
8b	12	0	11	12	12

TABLE 3. Antimicrobial activities of (1) and (3) at different concentrations .

Tested samples	Concentration (µg)	Inhibition zone (mm) of				
		Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Candida albicans*
1	210	0	17	20	25	30
	105	0	17	20	25	29
	50	0	16	20	25	29
	25	0	15	17	25	25
	12.5	0	12	15	25	25
	6	0	9	12	22	22
	3	0	0	0	20	19
	1.5	0	0	0	10	10
	0.75	0	0	0	0	0
3	210	17	23	20	27	29
	105	15	20	20	27	29
	50	12	15	15	25	25
	25	0	10	0	17	20
	12.5	0	0	0	15	15
	6	0	0	0	10	10
	3	0	0	0	0	0
Reference antibiotic Streptomycin sulphate	1000	30	40	40	0	37
	500	25	30	34	0	30
	250	20	20	25	0	22
	125	0	13	0	0	0
	60	0	0	0	0	

TABLE 4 . Combination of streptomycin sulphate at 125 + samples at 15 ug.

Treatment	Concentration ug	Inhibition zone (mm) of				
		Escherichia coli	Staphylococcus aureus	Pseudomonas aeruginosa	Bacillus cereus	Candida albicans
Antibiotic	125	0	13	0	0	0
1	15	0	0	0	0	0
3	15	0	0	0	0	20
8a	420	0	0	0	0	0
8b	420	0	0	0	0	0
1+ anti	15+125	0	0	0	0	0
3+ anti	15+125	0	0	15	0	20
8a+ anti	15+125	0	0	20	0	15
8b+ anti	15+125	0	0	15	0	13

Conclusions

Unfurtionatilly, the solvent molecules was included in the reaction and reacted with the biological impotent amino group and consequently decrease the biological effect of the product, which was unexpected result from the plant molecular design. All attempts to avoid this by changing the solvent had been failed.

Acknowledgement

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تحضير مشتقات الانديوكينوكسالين و النمذجة الجزيئية والتقييم البيولوجي لها كمضادات للفطريات والبكتيريا

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تحضيرمشتقات ثيوكربوهيدرازون للانديوكينوكسالين بنواتج جيدة.تم كذلك تم إثبات التراكيب الكيميائية عن طريق التحليلات الطيفية المختلفة ثم اجراء النمذجه الجزيئيه لحساب كل من التركيب الأمثل والأطياف الاهتزازية لبعض المركبات المدروسة. تم الحصول على نتائج واعدة كمضادة للميكروبات من المركبات الجديدة.