Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(4):192-201
(http://derpharmachemica.com/archive.html)

Synthesis of hexahydro-6*H*-indolo[2,3-*b*]quinoxaline derivatives as potential antibacterial and anti-inflammatory agents

Hajjaj H. M. Abdu-Allah[1], Samia G. Abdel-Moty[1] and Helal F. Heta[2]

[1]*Department Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut-71526, Egypt*
[2]*Department of Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut 71526, Egypt*

ABSTRACT

*In this study, hexahydro-6H-indolo[2,3-b]quinoxaline (**1**) was synthesized by condensation of isatin with 1,2-diaminocyclohexane. It was then reacted with methyl bromoacetate, hydrazine and finally condensed with selected carbonyl compounds to give the hydrazones **4-23**. The new compounds have been characterized, screened for biological activity. Some of them showed significant antibacterial and anti-inflammatory activities. In particular, compound **20** showed interesting activities as anti-inflammtory and antibacterial.*

Key words: hexahydro-6*H*-indolo[2,3-*b*]quinoxaline, carbohydrazide, hydrazone, isatin, E/Z

INTRODUCTION

Heterocyclic compounds containing quinoxaline and/or indole rings have attracted considerable attention as a consequence of their diverse biological activities. Quinoxalines are the active moieties of many antibiotics such as echinomycin, levomycin and actinoleutin [1]. The antimicrobial potency of quinoxalines is attributed to the prevention of DNA-directed RNA synthesis by virtue of binding to CpG site on DNA [2].

In particular, 6H-indolo[2,3-b]quinoxalines exhibit a number of biological activities including; cytotoxicity [3], antiviral activity [4], antimicrobial [5] and anti-inflammatory [6]. Moreover, some indoloquinoxaline derivatives substituted at nitrogen of indole moiety had been described as antibacterial and anti-inflammatory agents [6]. Similarly, indoloquinolines were found to exhibit antibacterial activity towards methicillin-resistant staphylococcus aureus (anti-MRSA activity) [7]. Also, Indolo[2,1-b]quinazoline-6,12-dione was used as a versatile lead for designing potential drugs with diverse medical functions [8]. On the other hand, 11*H*-indeno[1,2-*b*]quinoxalin-11-one oxime and several related analogs inhibited the production of pro-inflammatory cytokines and nitric oxide (NO) by LPS-stimulated monocytes/macrophages and peripheral blood mononuclear cells (PBMCs) [9]. Currently the synthesis of partially saturated fused heterocyclic compounds is of interest. A library of hexahydropyrroloindoles (HPI) [10] and tetrahydroindolo[2,3-a]quinolizines were generated and submitted for various biological screenings [11]. Based on the versatile biological activity of quinoxaline and indole we are interested in designing new flexible fused analogs of these two rings. Finding drug with both anti-inflammatory and antimicrobial activities is highly desirable from many aspects, safety, efficacy, pharmacoeconomic and patient compliance. Accordingly, the present work aims to synthesize the new hexahydroindoloquinoxaline derivatives, elucidation of their structures and testing their anti-inflammatory and antimicrobial activities.

MATERIALS AND METHODS

Chemistry

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, SMP3, Staffordshire, UK) and were uncorrected. Pre-coated silica gel plates (Kieselgel 0.25 mm, 60G F254, Merck, Darmstadt, Germany) were used for TLC monitoring of reactions using hexane/ethyl acetate (5:1) as mobile phase. Visualization of the spots was effected using an ultraviolet lamp (Spectroline, model CM-10, Seattle, USA) (λ= 254 nm). IR spectra were carried out as KBr discs on a Shimadzu IR-470 Spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 MHz spectrometer (Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt) or on a Varian EM-360L NMR spectrometer (60 MHz, Varian, CA, USA) at Faculty of Pharmacy, Assiut University, Assiut, Egypt. Chemical shifts are expressed in δ -values (ppm) relative to tetramethylsilane (TMS) as an internal standard using DMSO-d6 as a solvent and deuterium oxide was used for the detection of exchangeable protons. Elemental microanalyses were performed on a Vario elemental analyzer III (Vario, Hanau, Germany) at the unit of Microanalysis, Faculty of Science, Cairo University.

1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxaline (1) A mixture of isatin (10 g, 0.068 mol), 1,2-cyclohexanediamine (7.76 g, 0.068 mol) and glacial acetic acid (60 mL) was heated under reflux for 6 h. Precipitate formed after reaction mixture cooling and adding water was collected and recrystallized from ethanol. Yield: 85% (13.02 g); m.p. 258 ^{o}C [12]. 1H NMR (60 MHz, DMSO-d6) *δ* 9.02 (br, 1H, NH), 7.46 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.27 (d, *J* = 7.6 Hz, 1H, Ar-H), 6.85 (m, 2H, Ar-H), 3.17(m, 2H, CH), 2.27 (m, 1H, CH), 1.99 (m, 1H, CH), 1.74 (m, 2H, CH_2), 1.43 (m, 2H, CH_2), 1.35 (m, 2H, CH_2).

Methyl 1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxaline-6-acetate (2)

A mixture of **1** (5 g, 0.022 mol), anhydrous potassium carbonate (3 g, 0.022 mol), methy bromoacetate (1 ml, 0.022 mol) in *acetone* (40 ml) was stirred overnight in a round bottomed flask. The reaction mixture was cooled, evaporated under vacuum, taken with water, filtered, washed with water and dried. It was recrystallised from ethanol as yellow crystals (5.5 g, 83%),

IR (KBr) ν: 3035, 2910, 1740, 1640, 1620, 1550, 1475, 1450, 1420, 1393, 1307, 1250, 1187, 1112, 1002, 975, 742 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) *δ:* 8.20 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.80-7.13 (m, 3H, Ar-H), 5.30 (s, 2H, CH_2), 3.70 (s, 3H, OCH_3), 3.30-2.75 (m, 6H, CH and CH_2), 2.20-1.60 (m, 4H, CH_2).

1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxaline-6-acetohydrazide (3)

2 (5 g, 0.017 mol) and hydrazine hydrate (99%, 0.068 mol) into 30 mL of absolute ethanol in a 50 mL round bottomed flask. The flask was heated under reflux for 8 h. Cooling, filteration washing with ethanol and recrystallized from hot dioxane. Yield 94% and Mp = 221-222.5 ^{o}C. IR (KBr) ν: 3400, 3260, 3035, 2910, 1644, 1614, 1533, 1482, 1454, 1416, 1393, 1307, 1187, 1112, 1002, 975, 742 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 9.42 (s, 1H, NH), 8.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.57 (d, 2H, *J* = 4.0 Hz, 1H, Ar-H), 7.34-7.30 (m, 1H, Ar-H), 5.02 (s, 2H, CH_2), 4.29 (br. s, NH_2), 3.40-3.20 (m, 2H, CH), 3.10-3.00 (m, 4 H, CH_2), 2.10-1.90 (m, 4H, CH_2); ^{13}C (100 MHz, DMSO-d6) δ (ppm): 166.87, 148.2, 144.7, 144.15, 141.55, 133.21, 128.63, 120.96, 119.55, 110.92, 42.73, 32.69, 32.09, 23.17, 22.97. Anal. found C, 64.20; H, 6.25; N, 23.36 (%). Calc. for ($C_{16}H_{19}N_5O$): C, 64.63; H, 6.44; N, 23.55 (%).

General method for the synthesis of compounds (4-23)

In a round bottom flask containing **3** (0.25 g, 0.8 mmol) in ethyl alcohol (5 ml) was added un/substituted carbonyl compounds (0.82 mmol) and few drops of acetic acid. The mixture was stirred with heating under reflux overnight. Progress of the reaction was checked with TLC (hexane/ethyl acetate 4/1). The precipitate formed was filtered while hot, washed with cold ethanol and recrystallized from dioxane to give the target compounds from (60-80%).

N'-benzylidene-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(4)

80% yield, m.p: 286-287 ^{o}C. IR (KBr) ν: 3450, 3165, 2910, 1670, 1608, 1554, 1483, 1455, 1408, 1306, 1271, 1188, 1116, 739, 690 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 12.74 (s, 1H, NH), 11.90 (s, 0.3H, NH), 8.19 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.09 (s, 1H, CH=), 7.78 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.69 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.66-7.58 (m, 1H, Ar-H), 7.50 -7.46 (m, 5H, Ar-H), 7.37-7.30 (m, 1H, Ar-H), 5.64 (s, 2H, CH_2), 5.25 (s, 0.6H, CH_2), 3.50-3.30 (m, 2H, CH), 3.09-3.02 (m, 4H, CH_2), 2.00-1.80 (m, 4H, CH_2); ^{13}C (100 MHz, DMSO-d6) δ (ppm): 168.89, 148.26,

148.26, 144.75, 144.67, 144.24, 141.91, 134.45, 133.22, 130.52, 129.31, 128.70, 127.58, 127.49, 120.91, 119.44, 111.11, 42.86, 32.68, 32.10, 23.17, 22.96. Anal. found C, 71.38; H, 5.73; N, 18.08 (%). Calc. for ($C_{23}H_{23}N_5O$): C, 71.67; H, 6.01; N, 18.17 (%).

N'-(2-Hydroxybenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(5)
60% yield, m.p: 301-302 ^oC. IR (KBr) ν: 3415, 3170, 3060, 2910, 1666, 1605, 1557, 1480, 1455, 1408, 1307, 1281, 1188, 1114, 742, 647 cm^{-1}; ^1H NMR (400 MHz, DMSO-d6) δ (ppm): 12.07 (s, 1H, OH), 11.66 (s, 1H, OH), 10.94 (s, 1H, NH), 10.10 (s, 1H, NH), 8.47 (s, 1H, CH=), 8.39 (s, 1H, CH=), 8.20-8.18 (m, 2H, Ar-H), 7.80 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.68 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.58-7.55 (m, 3H, Ar-H), 7.34-7.27 (m, 5H, Ar-H), 6.94-6.89 (m, 5H, Ar-H), 5.61 (s, 1.3H, CH_2), 5.24 (s, 0.7 H, CH_2), 3.50-3.30 (m, 2H, CH), 3.20-3.00 (m, 4H, CH_2), 2.00-1.90 (m, 4H, CH_2); ^{13}C (100 MHz, DMSO-d6) δ (ppm): 168.54, 164.11, 157.75, 156.92, 148.33, 148.25, 148.03, 144.96, 144.73, 144.24, 142.03, 141.91, 141.69, 133.28, 133.21, 131.98, 131.77 129.63, 128.78, 128.68, 126.85, 121.13, 121.03, 120.92, 120.89, 120.59, 119.89, 42.81, 32.10, 23.17, 23.14, 22.95. Anal. found C, 69.06; H, 6.00; N, 17.23 (%). Calc. for ($C_{23}H_{23}N_5O_2$): C, 68.81; H, 5.77; N, 17.44 (%).

N'-(4-Methoxybenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(6)
70% yield, m.p: 271-272 ^oC. IR (KBr) ν: 3415, 3160, 3060, 2915, 1668, 1599, 1556, 1501, 1455, 1408, 1305, 1250, 1161, 1116, 1025, 822, 736 cm^{-1}; ^1H NMR (60 MHz, DMSO-d6) *δ:* 11.90 (br. s, 1H, NH), 8.46-7.94 (m, 1 H, Ar-H), 7.90-6.80 (m, 3H, Ar-H), 5.55 (s, 11/2H, CH_2), 5.17 (s, 1/2 H, CH_2), 3.80 (s, 3H,OCH_3), 3.40-2.70 (m, 6H, CH and CH_2), 2.25-1.68 (m, 4H, CH_2). Anal. found C, 69.69; H, 5.77; N, 17.04 (%). Calc. for ($C_{24}H_{25}N_5O_2$): C, 69.38; H, 6.06; N, 16.86 (%).

N'-(4-Methylbenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(7)
75% yield, m.p: 274-275 ^oC. IR (KBr) ν: 3565, 3160, 3040, 2910, 1669, 1607, 1554, 1485, 1454, 1407, 1306, 1271, 1188, 1115, 926, 804, 737 cm^{-1}; ^1H NMR (60 MHz, DMSO-d6) *δ:* 12.10 (br. s, 1H, NH), 8.45-8.00 (m, 1 H, Ar-H), 7.90-7.00 (m, 3H, Ar-H), 5.67 (s, 11/2H, CH_2), 5.27 (s, 1/2 H, CH_2), 3.40-2.85 (m, 6H, CH and CH_2), 2.30-1.67 (m, 4H, CH_2). Anal. found C, 71.95; H, 5.99; N, 17.84 (%). Calc. for ($C_{24}H_{25}N_5O$): C, 72.16; H, 6.31; N, 17.53 (%).

N'-(2-Methoxybenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(8)
70% yield, m.p: 290-292 ^oC. IR (KBr) ν: 3395, 3060, 2915, 1677, 1607, 1455, 1405, 1306, 1267, 1117, 736. cm^{-1}; ^1H NMR (60 MHz, DMSO-d6) δ(ppm): 11.90 (s, 1H, NH), 8.45 (s, 1H, CH), 8.37-8.10 (d, 1H, Ar-CH), 8.07-7.85 (d, 1H, Ar-CH), 7.80-6.80 (m, 3H, Ar-CH), 5.60 (s, 11/2H, CH_2), 5.20 (s, 1/2 H, CH_2), 3.90 (s, 3H,OCH_3), 3.35-2.80 (m, 6H, CH and CH_2), 2.35-1.67 (m, 4H, CH_2). Anal. found C, 69.38; H, 6.06; N, 16.86 (%). Calc. for ($C_{24}H_{25}N_5O_2$): C, 69.38; H, 6.06; N, 16.86 (%).

N'-(4-Dimethylaminobenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)aceto hydrazide (9)
68% yield, m.p: 231.5-233.5 ^oC. IR (KBr) ν: 3395, 3060, 2915, 1677, 1607, 1455, 1405, 1306, 1267, 1117, 736 cm^{-1}; ^1H NMR (60 MHz, DMSO-d6) δ (ppm): 11.80 (s, 1H, NH), 8.50-8.17 (d, 1H, Ar-CH), 8.00 (s, 1H, CH), 7.90-7.15 (m, 4H,d, Ar-CH), 7.00-6.50 (d, 2H, m, Ar-CH), 5.68 (s, 11/2H, CH_2), 5.25 (s, 1/2 H, CH_2), 3.80-2.80 (m, 12H, CH and CH_2), 2.35-1.67 (m, 4H, CH_2). Anal. found C, 70.57; H, 7.10; N, 19.61 (%). Calc. for ($C_{25}H_{28}N_6O$): C, 70.07; H, 6.59; N, 19.61 (%).

N'-(3-Chlorobenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(10)
73% yield, m.p: 269-270 ^oC. IR (KBr) ν: 3470, 3055, 2910, 1671, 1609, 1455, 1407, 1306, 1278, 1115, 755 cm^{-1}; ^1H NMR (60 MHz, DMSO-d6) δ (ppm): 12.30 (s, 1H, NH), 8.33 (s, 1H, CH), 8.25-8.08 (d, 1H, Ar-CH), 8.00-7.18 (m, 7H, d, Ar-CH), 5.70 (s, 11/2H, CH_2), 5.3 (s, 1/2 H, CH_2), 3.60-2.80 (m, 6H, CH and CH_2), 2.35-1.70 (m, 4H, CH_2). Anal. found C, 65.73; H, 5.01; N, 17.01 (%). Calc. for ($C_{23}H_{22}ClN_5O$): C, 65.79; H, 5.28; N, 16.68 (%).

N'--(Benzo[d][1,3]dioxol-5-ylmethylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide (11)
75% yield, m.p: 300-302 ^oC. IR (KBr) ν: 3440, 3160, 3050, 2905, 1669, 1609, 1555, 1480, 1459, 1405, 1307, 1277, 1250, 1188, 1116, 1029, 925, 792, 734 cm^{-1}. ^1H NMR (60 MHz, DMSO-d6) δ (ppm): 11.87 (s, 1H, NH), 8.50-7.80 (m, 2H, Ar-CH,CH), 7.65-6.75 (m, 6H, Ar-CH), 6.05 (s, 11/2H, CH_2), 5.55 (s, 1/2 H, CH_2), 3.66-2.67 (m, 10H, CH and CH_2), 2.20-1.50 (m, 4H, CH_2). Anal. found C, 70.50; H, 6.11; N, 16.38 (%). Calc. for ($C_{25}H_{25}N_5O_2$): C, 70.24; H, 5.89; N, 16.38 (%).

N'-(4-hydroxybenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(12)
62% yield, m.p: 328-330 ^{o}C. IR (KBr) ν: 3550, 3160, 3050, 2910, 1670, 1599, 1525, 1503, 1456, 1409, 1405, 1309, 1260, 1223, 1189, 1156, 1117, 821, 739 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 11.67 (br. s, 2H, NH, OH), 8.25 (br. d, 2H, Ar-CH), 8.00 (s, H, CH), 7.90-7.10 (m, 4H, Ar-CH), 7.07-6.5 (br. d, 2H, Ar-H), 5.60 (s, 11/2H, CH_2), 5.20 (s, 1/2 H, CH_2), 3.50-2.70 (m, 6H, CH and CH_2), 2.25-1.60 (m, 4H, CH_2). Anal. found 68.51; H, 6.11; N, 17.70 (%). Calc. for ($C_{23}H_{23}N_5O_2$): C, 68.81; H, 5.77; N, 17.4 (%).

N'-(3,4-Dimethoxybenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide (13)
78% yield, m.p: 305-306 ^{o}C. IR (KBr) ν: 3445, 3250, 3050, 2915, 1668, 1596, 1562, 1503, 1454, 1409, 1405, 1306, 1253, 1210, 1153, 1156, 1131, 1018, 732 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 12.00 (br. s, 1H, NH), 8.25 (br. d, 1H, Ar-CH), 8.03 (s, H, CH), 7.90-6.90 (m, 6H, Ar-CH), 5.67 (s, 1.5H, CH_2), 5.20 (s, 0.5H, CH_2), 3.9 (s, 6H, OMe), 3.50-2.80 (m, 6H, CH and CH_2), 2.35-1.65 (m, 4H, CH_2). Anal. found C, 67.07; H, 5.88; N, 15.71 (%). Calc. for ($C_{25}H_{27}N_5O_3$): C, 67.40; H, 6.11; N, 15.72 (%).

N'-(3,4,5-Trimethoxybenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)aceto hydrazide (14)
80% yield, m.p: 298-299.5 ^{o}C. IR (KBr) ν: 3445, 3170, 3050, 2910, 1669, 1609, 1569, 1486, 1457, 1405, 1308, 1273, 1227, 1153, 1117, 999, 729 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 12.10 (br. s, 1H, NH), 8.35-8.10 (m, 1H, Ar-CH), 8.00 (s, H, CH), 7.85-6.80 (m, 5H, Ar-CH), 5.65 (s, 1.5H, CH_2), 5.20 (s, 0.5H, CH_2), 3.9 (s, 6H, OMe), 3.75 (s, 3H, OMe), 3.40-2.70 (m, 6H, CH and CH_2), 2.25-1.65 (m, 4H, CH_2). Anal. found C, 65.66; H, 5.95; N, 13.81 (%). Calc. for ($C_{26}H_{29}N_5O_4$): C, 65.67; H, 6.15; N, 13.46 (%).

N'-(4-Nitrobenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(15)
70% yield, m.p: 315-317 ^{o}C. IR (KBr) ν: 3490, 3170, 3055, 2920, 1682, 1606, 1575, 1503, 1400, 1405, 1327, 1257, 1209, 1153, 1120, 821, 761 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 11.80 (br. s, 1H, NH), 8.30 (s, H, CH), 8.20-7.00 (m, 8H, Ar-CH), 5.65 (s, 1.5H, CH_2), 5.20 (s, 0.5H, CH_2), 3.35-2.65 (m, 6H, CH and CH_2), 2.15-1.50 (m, 4H, CH_2). Anal. found C, 63.93; H, 5.43; N, 19.73 (%). Calc. for ($C_{23}H_{22}N_6O_3$): C, 64.17; H, 5.15; N, 19.52 (%).

N'-(3-Nitrobenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(16)
73% yield, m.p: 299-300 ^{o}C. IR (KBr): 3475, 3165, 3055, 2919, 1673, 1626, 1521, 1455, 1405, 1333, 1276, 1137, 731 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 12.30 (br. s, 1H, NH), 8.50 (s, H, CH), 8.40-7.90 (m, 4H, Ar-CH), 7.87-7.10 (m, 4H, Ar-CH), 5.65 (s, 1.5H, CH_2), 5.25 (s, 0.5H, CH_2), 3.35-2.80 (m, 6H, CH and CH_2), 2.25-1.60 (m, 4H, CH_2). Anal. found C, 64.18; H, 5.35; N, 19.33 (%). Calc. for ($C_{23}H_{22}N_6O_3$): C, 64.17; H, 5.15; N, 19.52 (%).

N'-(2-thienylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(17)
70% yield, m.p: 292.5-294 ^{o}C. IR (KBr) ν: 3450, 3165, 3055, 2910, 1669, 1611, 1555, 1484, 1455, 1409, 1306, 1277, 1187, 1116, 926, 833, 781, 734, 698 cm^{-1}. ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 11.80 (br. s, 1H, NH), 8.47 (s, H, CH), 8.35-8.00 (m, 3H, Ar-CH), 7.75-6.90 (m, 4H, Ar-CH), 5.50 (s, 1.5H, CH_2), 5.17 (s, 0.5H, CH_2), 3.35-2.60 (m, 6H, CH and CH_2), 2.10-1.50 (m, 4H, CH_2). Anal. found C, 64.85; H, 5.78; N, 17.89 (%). Calc. for ($C_{21}H_{21}N_5OS$): C, 64.43; H, 5.41; N, 17.89 (%).

N'-(4-Chlorobenzylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(18)
68% yield, m.p: 278-279 ^{o}C. IR (KBr) ν: 3425, 3235, 3055, 2910, 1670, 1623, 1552, 1478, 1456, 1404, 1303, 1271, 1116, 813, 731 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 11.85 (br. s, 1H, NH), 8.25 (s, H, CH), 8.20-7.95 (d, 1H, Ar-CH), 7.90-7.10 (m, 7H, Ar-CH), 5.55 (s, 1.5H, CH_2), 5.15 (s, 0.5H, CH_2), 3.35-2.70 (m, 6H, CH and CH_2), 2.20-1.65 (m, 4H, CH_2). Anal. found C, 65.61; H, 5.14; 16.64 (%). Calc. for ($C_{21}H_{21}N_5OS$): C, 65.79; H, 5.28; 16.68 (%).

N'-(2-pyridenylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(19)
75% yield, m.p: 231.5-232.5 ^{o}C. IR (KBr) ν: 3445, 3155, 3015, 2855, 1670, 1608, 1554, 1456, 1416, 1394, 1307, 1226, 1200, 1140, 1074, 1002, 952, 784, 734 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 12.25 (br. s, 1H, NH), 8.55- (br. d, H, CH), 8.20-7.20 (m, 8H, Ar-CH, CH=), 7.90-7.10 (m, 7H, Ar-CH), 5.67 (s, 1.5H, CH_2), 5.27 (s, 0.5H, CH_2), 3.40-2.75 (m, 6H, CH and CH_2), 2.30-1.55 (m, 4H, CH_2). Anal. found C, 68.23; H, 5.38; N, 21.88 (%). Calc. for ($C_{22}H_{22}N_6O$): C, 68.38; H, 5.74; N, 21.75 (%).

N'-(2-oxoindolin-3-ylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide (20)
80% yield, m.p: 272.5-273.5 ^{o}C. IR (KBr) ν: 3500, 3205, 3040, 2915, 1720, 1684, 1607, 1550, 1480, 1455, 1313, 1229, 1194, 1143, 1121, 784, 745, 649 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 13.18 (br. s, 1H, OH), 12.70 (br. s, 1H, OH), 11.30 (br. s, 1H, NH), 8.19 (d, *J* = 7.5 Hz, 1H, Ar-H), 8.19 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.75 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.60-7.32 (m, 4H, Ar-H), 7.20-6.90 (m, 2H, Ar-H), 5.79 (s, 2H, CH_2), 5.46 (s, 0.4H, CH_2), 3.60-3.20 (m, 2H, CH), 305-2.75 (m, 4H,CH_2), 2.25-1.65 (m, 4H, CH_2); ^{13}C (100 MHz, DMSO-d6) δ (ppm): 162.95, 148.39, 145.09, 144.04, 143.09, 141.69, 133.29, 132.33, 128.80, 123.08, 121.40, 121.02, 120.04, 119.55, 111.67, 111.10, 32.65, 32.09, 23.12, 22.91. Anal. found C, 67.66; H, 5.20; N, 19.90 (%). Calc. for ($C_{24}H_{22}N_6O_2$): C, 67.59; H, 5.20; N, 19.71.

***N'*-(1-(phenyl)ethylidene)- 2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)acetohydrazide(21)**
75% yield, m.p: 288-289 ^{o}C. IR (KBr) ν: 3495, 3165, 3040, 2915, 1668, 1607, 1554, 1487, 1455, 1400, 1305, 1226, 1188, 1115, 825, 740 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 11.30 (br. s, 1H, NH), 8.20 (br. d, 1H, Ar-CH), 8.10-7.10 (m, 7H, Ar-CH), 5.67 (br. s, 2H, CH_2), 3.55-2.70 (m, 6H, CH and CH_2), 2.35 (s, 3H, CH_3), 2.20-1.55 (m, 4H, CH_2). Anal. found C, 72.49; H, 6.57; N, 17.21 (%). Calc. for ($C_{24}H_{25}N_5O$): C, 72.16; H, 6.31; N, 17.53.

***N'*-(1-(4-Fluorophenyl)ethylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)aceto hydrazide (22)**
77% yield, m.p: 218.5-219.5 ^{o}C. IR (KBr) ν: 3495, 3165, 3040, 2915, 1668, 1607, 1554, 1487, 1455, 1400, 1305, 1226, 1188, 1115, 825, 740 cm^{-1}; ^{1}H NMR (60 MHz, DMSO-d6) δ (ppm): 12.20 (br. s, 1H, NH), 8.35 (s, H, CH), 8.17 (d, 1H, Ar-CH), 8.10-7.10 (m, 7H, Ar-CH), 5.70 (s, 1.5H, CH_2), 5.27 (s, 0.5H, CH_2), 3.40-2.80 (m, 6H, CH and CH_2), 2.30-1.65 (m, 4H, CH_2). Anal. found C, 68.58; H, 5.19; N, 17.08 (%). Calc. for ($C_{23}H_{22}FN_5O$): C, 68.47; H, 5.50; N, 17.36.

***N'*-(1-(4-Methoxyphenyl)ethylidene)-2-(1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxalin-6-yl)aceto hydrazide (23)**
72% yield, m.p: 287-289 ^{o}C. IR (KBr) ν: 3405, 3155, 3040, 2905, 1676, 1594, 1560, 1458, 1410, 1400, 1309, 1268, 1206, 1118, 1035, 843, 743 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 10.96 (br. s, 1H, NH), 8.19 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.68 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.65-7.36 (m, 5H, Ar-CH), 7.10-6.90 (m, 1H, Ar-CH), 5.67 (s, 1.5H, CH_2), 5.35 (s, 0.5H, CH_2), 3.77 (s, 3H, OCH3), 3.50-3.30 (m, 2H, CH), 3.15-2.95 (m, 4H, CH_2), 2.37 (s, 3H, CH_3), 2.05-1.80 (m, 4H, CH_2); ^{13}C (100 MHz, DMSO-d6) δ (ppm): 169.72, 159.79, 149.03, 148.25, 144.71, 144.28, 141.93, 139.96, 133.21, 129.98, 128.67, 120.93, 120.88, 119.42, 119.23, 115.19, 112.27, 111.13, 56.49, 55.65, 43.23, 32.67, 32.10, 23.16, 22.95, 19.02, 14.30. Anal. found C, 69.99; H, 6.06; N, 16.52 (%). Calc. for ($C_{23}H_{22}FN_5O$): C, 69.91; H, 6.34; N, 16.31.

Biological screening

Anti-inflammatory activity

The rat paw thickness was measured with a Vernier calliper (SMIEC, Shangahai, China). Carrageenan (Sigma, USA), indomethacin (Liometacin® vial, Nile Company, Cairo, Egypt), and normal saline (Almottahedoon Pharma Company, Cairo, Egypt) were obtained from the local market. Male adult albino rats (120–150 g) were obtained from the animal house (Faculty of Medicine, Assiut University, Egypt). Animals were housed in separate cages 6 animals each, at 25±2°C. Animals were allowed free access to rodent chow and water and maintained at a 12 h light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines [13] and Institutional Ethical Committee Approval was obtained. The anti-inflammatory activity of the newly synthesized compounds **3-23** were evaluated according to the carrageenan induced paw edema method in comparison with indomethacin as a reference drug [14]. The test is based on pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 ml of 1 % solution in normal saline) into the right hind paw of the rats. Male adult albino rats were divided into groups of four animals each. The rat paw thickness was measured before and 1 h after carrageenan injection to detect the carrageenan induced inflammation. Test compounds **3**-**6**, **9**-**20**, **23** and indomethacin at a dose of 0.02 mmol/Kg were suspended in 1 % NaCMC in normal saline. Suspensions were injected i.p. (1 ml each) to rats 1 h after carrageenan injection. In addition, a control group received the vehicle 1 % NaCMC solution in normal saline (negative control).

The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 h after injection of the test compounds, reference drug, and control and results were listed in Table 1

The percentage of edema and percentages of edema inhibition were calculated according to the following

$$\% \text{ Edema inhibition} = \frac{(V_R\text{-}V_L)\text{control} - (V_R\text{-}V_L)\text{treated}}{(V_R\text{-}V_L)\text{control}} \text{ X } 100$$

Where, VR: Average right paw thickness, VL: Average left paw thickness.

Antibacterial testing

The antibacterial activity of all the target compounds was investigated in-vitro against methicillin resistant Staphylococcus aureus (MRSA), Escherichia coli, and Klebsiella pneumoniae (clinical isolates obtained from Infection Control Unit, Assiut University Hospital, Faculty of Medicine, Assiut University) using agar cup diffusion method [15] for susceptibility screening, and twofold dilution method [16] for MIC determination. Gentamycin was used as a reference drug, and DMSO was used as a solvent control.

Agar cup diffusion method

38 Grams of Mueller-Hinton agar medium (MH) (Hi-Media, M 001) were added to 1 L of distilled water, heated to boiling to dissolve the ingredients completely, and sterilized by autoclaving at 121°C for 30 minutes. High density inocula were made by diluting 3-5 well isolated colonies grown overnight on selective media in 5 mL of distilled water to prepare a suspension equivalent in density to 0.5 McFarland Barium Sulfate standard unit with average turbidity 10^8 CFU/mL [23]. The sterile Petri dishes were seeded with 100 µL of the microorganism; a specified amount of the molten MH agar medium (45-50 °C) was poured into the seeded Petri dishes to give a depth of 3-4 mm and allowed to solidify. Cylindrical plugs were removed from the agar using sterile cork borer. One hundred µL of each of the tested compounds or gentamycin (20 mg/mL in DMSO), or the blank solvent, were added to the wells in triplicate. The seeded plates were incubated at 37 °C for 24 hrs then the average diameters of the inhibition zones were measured in millimeters.

Minimum inhibitory concentration

The MIC was determined using twofold dilution method [16] for compounds having moderate to strong antibacterial activity. The squares of inhibition zone diameters were plotted against log concentrations of the tested compounds, extrapolation of the resulting straight line to intersect with log concentration scale in the curve corresponded to log MIC, and MIC was obtained as antilog [17].

RESULTS AND DISCUSSION

Chemistry

1,2,3,4,4a,11a-hexahydro-6H-indolo[2,3-b]quinoxaline (**1**) was synthesized similar to the synthesis of 6*H*-indolo[2,3-*b*]quinoxaline but using 1,2-diaminocyclohexane instead of *o*-phenylenediamine. There is only one report for the synthesis of compound **1** without derivatization [12]**.** We are interested in the synthesis of this compound and its derivatives. It was reacted with methyl bromoacetate to give **2** that was reacted with hydrazine to give the key intermediate acetohydrazide (**3**). Refluxing **3** with different aldehydes, acetophenones and isatins provided the target compounds (**4-23**). All of the new compounds have been characterized by ^{1}H NMR, elemental analysis and some by ^{13}C NMR. Due to geometric isomerism with respect to the imino group (E, Z isomers) and rotational isomerism as a result of hindered rotation about the amide linkage (*anti/syn* -conformers), hydrazones can exist as four isomers. In DMSO solutions acylhydrazones form mostly (up to 100%) E –isomers [18,19] and the *syn*- conformers are dominant [20-23]. Most signals in the ^{1}H NMR spectra of hydrazones **4-23** are observed as multiplets or as two overlapped doublets. Due to the *anti/syn* – isomerism.

The new hydrazones **4-23** exist as a mixture of two conformers (*syn/anti/*), as exemplified by doubled proton resonances assigned to the CH_2CO, =CH and NH groups in 1H NMR spectra. The proton signal assigned to the *anti* -conformer CH_2CO is upfield as compared with the signal of the *syn* - conformer. The percentages of *anti* - and *syn* – conformers (1/2) are calculated from the ratio of integral intensities of these signals. However, the percentage of

anti – conformer of hydrazone prepared from 2-hydroxybenzaldehyde has increased compared to that of hydrazones obtained from benzaldehydes [20,21]. It was suggested that the formation of intramolecular hydrogen bonds with the participation of the imino nitrogen lone electron pair and the proton of the 2-hydroxy group is responsible for this effect [18,20].

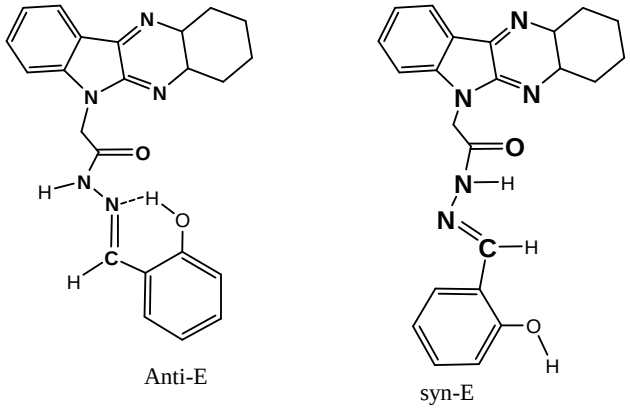
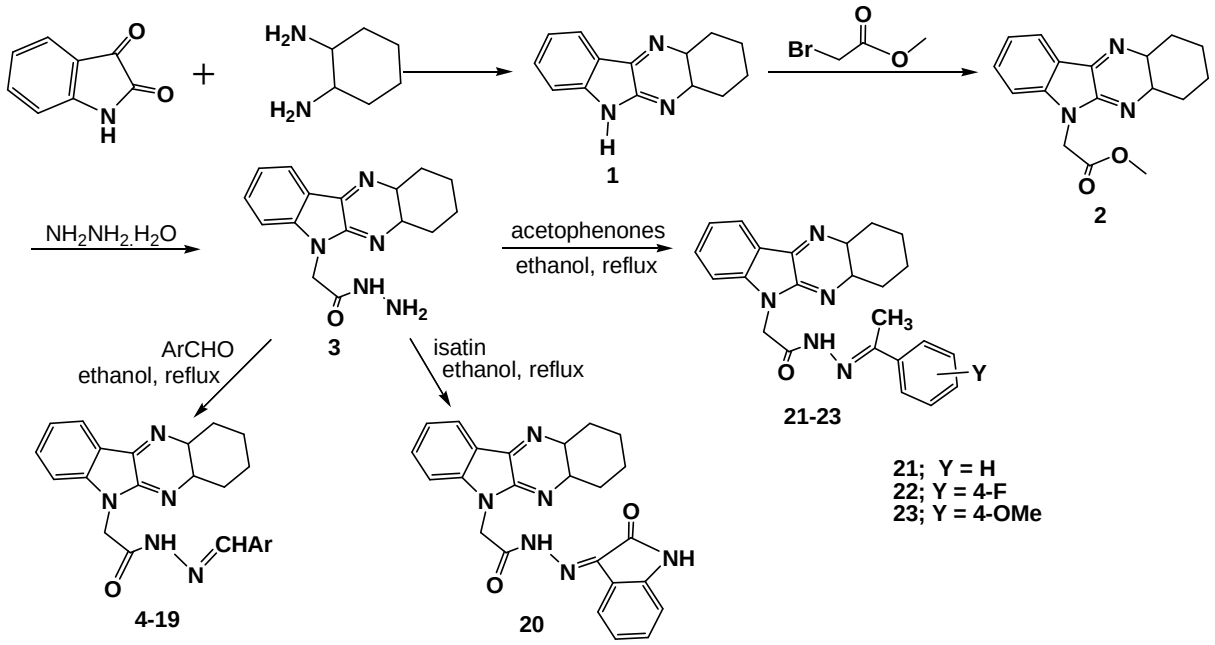


Figure 1. Anti- and syn- conformers of hydrazone containing 2-OH group



Ar = Ph (**4**); Ar = (2-OH)Ph (**5**); Ar = (*4*-OMe)Ph (**6**); Ar = (*4*-Me)Ph (**7**); Ar = (*2*-OMe)Ph (**8**); Ar = (4-$NHMe_2$)Ph (**9**); Ar = (3-Cl)Ph (**10**); Ar = (2,3-OCH_2O-)Ph (**11**), Ar = (4-OH)Ph (**12**); 13; Ar = (3,4-di-OMe)Ph (**13**); Ar = (3,4,5-tri-OMe)Ph (**14**); Ar = (4-NO_2)Ph (**15**); Ar = (3-NO_2)Ph (**16**); Ar = 2-thioenyl (**17**); Ar = (4-Cl)Ph (**18**); Ar = 2-pyridyl (**19**)

Biology

Anti-inflammatory activity

The *in vivo* anti-inflammatory activity was tested by carrageenan paw edema method using indomethacin as the reference drug. It is clear from the results (Table 1) that benzylidene derivative (**4**) showed better anti-inflammatory activity than the starting carbohydrazide (**3**).

Moreover, introducing 4-dimethylamino (**9**), 3,4-dimethoxy (**13**) or 4-chloro (**18)** at benzylidene moiety is tolerated and exhibited somewhat better activity. Unfortunately, we couldn't test the activity of **14** (3,4,5-trimethoxy) due to

poor solubility. Replacing benzylidene with isatin (**20**), has resulted in the most active compound in this series. Accordingly, four compounds of this series showed significant anti-inflammatory activity.

Moreover, introducing 4-dimethylamino (**9**), 3,4-dimethoxy (**13**) or 4-chloro (**18)** at benzylidene moiety is tolerated and exhibited somewhat better activity. Unfortunately, we couldn't test the Table 1. Percentage of edema inhibition of compounds **3-6**, **9-13**, **15-20** and **23** and indomethacin on carrageenan induced paw edema in rats activity of **14** (3,4,5-trimethoxy) due to poor solubility. Replacing benzylidene with isatin (**20**), has resulted in the most active compound in this series. Accordingly, four compounds of this series showed significant anti-inflammatory activity (**9**,**13**, **18** and **20**).

Compd. No.	Percentage of edema inhibition					
	1/2 h	1 h	2 h	3 h	4 h	5 h
Control	-	-	-	-	-	-
3	8	19	43	43	63	63
4	12	31	53	65	75	75
5	3	3	8	8	22	15
6	5	8	24	29	31	40
9	8	19	24	38	74	81
10	5	8	12	24	46	31
11	5	8	17	34	53	53
12	8	17	29	41	58	60
13	17	38	60	77	79	73
15	0	8	12	22	34	41
16	3	12	36	30	38	30
17	5	17	29	29	43	26
18	8	24	12	41	64	73
19	5	24	43	62	75	45
20	12	15	29	58	73	79
23	5	17	17	43	32	43
Indomethacin	12	27	46	72	81	83

Antibacterial activity

In vitro antibacterial activity of compounds **3-23** and gentamycin was determined by agar cup diffusion method and twofold dilution method against bacteria such as MRSA and Klebsiella pneumonia and E. Coli. The results are presented in Table 2. The key carbohydrazide (**3)** showed comparable activity to gentamycin against MRSA and Klebsiella pneumonia and better activity against E. coli.

Table 2. The antibacterial activity of compounds 3-23. Inhibition zone in mm and MICs in Umol/mL

	MRSA		**Klebsiella pneumonia**		**E.coli**	
	Inhibition zone (mm)	MIC µM/mL	Inhibition zone (mm)	MIC µM/mL	Inhibition zone (mm)	MIC µM/mL
Gentamycin	20	69	23	50	20	70
3	22	70	25	50	26	40
4	14	180	18	170	15	190
5	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
6	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
7	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
8	14	160	13	170	16	160
9	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
10	16	155	17	130	20	85
11	15	145	13	130	11	140
12	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
13	15	158	16	155	17	125
14	14	160	13	190	12	170
15	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
16	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
17	24	50	27	33	28	25
18	23	20	18	90	16	140
19	21	73	22	64	18	79
20	19	85	20	75	20	76
21	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
22	18	67	21	80	19	78
23	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve

Unexpectedly, the benzylidene derivatvies (**4**-**17**) showed low or even no activity. Replacing benzylidene with thienyl group (**17**), provided the highest activity against the three strains even in comparison with the reference drug (gentamycin). On the other hand, replacing benzylidene

with pyridyl (**19**), isatin (**20**) or 4-fluoroacetophenone (**22**) provided compounds with comparable activity to that of the reference drug. Accordingly, compound (**17)** showed the highest antibacterial activity but no anti-inflammatory activity, while compound (**20)** showed the highest anti-inflammatory activity and significant antibacterial activity.

CONCLUSION

hexahydroindoloquinoxalines have been synthesized (**2**-**23**), characterized and screened for anti-inflammatory and bacterial activities. Four compounds showed significant anti-inflammatory activity (**4**, **9**, **18** and **20**) and antibacterial activity (**17**, **19**, **20** and **22**). Compound (**20)** showed significant activity and is a good candidate for developing drug with anti-inflammatory antibacterial activities.

Acknowledgements
This work was supported by Faculty of Pharmacy, Assiut University.

REFERENCES

[1] P. G Davey “Antimicrobial Chemotherapy” in “Concise Textbook of Medicine”, Oxford University press: **2000,** 1475.
[2] A. S. Khosarkar, D. B. Shinde, *Bioorg. Med. Chem. Lett.* **2006**, 16, 6181.
[3] (a) S. S. Karki, R. Hazare, S. Kumar, V. S. Bhadauria, J. Balzarini and E. D. Clercq, Acta. pharma, **2009**, 59, 431; (b) C. D. Smith, C. B. Mayers, J. T. Zilfour, S. N. Smith and D. S. Lawrence, Oncol. Res, **2000**, 12 219. (c) N. S. Moorthy, C. Karthikeyan and P.Trivedi, *J. Enzy, Inhib. Med. Chem.* **2010**, 25, 394. (d) K. M. Driller, S. Libnow, M. Heim, M. Harms, K. Wend, M. Lalk, D. Michalik, *Org. Biomed. Chem*, **2008**, 6, 4218.
[4] (a) J. Harmenberg, B. wahren, J. Bergman, S. Akerfeldt and L. Lundblad, *Antimicrob. Agents chemotherp*, **1988**, 32, 1720. (b) L. M. Wilhelmson, N. Kingi, J. Bergman, *J. Med. chem*, **2008**, 51, 7744. (c) N. Patel, J. Bergman and A. Graslund, *Nuc. Nuc. Nuc. Acids*, **1991**, 10, 699. (d) M. O. Shibinskaya, S. A. Lyakhov, A. V. Mazepa, S. A. Andronati, A. V. Turov, N. M. Zholobak, N. Y. Spivak, *Eur. J. Med. Chem*, **2010**, 45, 1237.
[5] G. Gupta, P. Verma, *Chem Sci Trans.*, **2014**, 3, 876-884
[6] (a) J. L. Thomas, O. S. Rajesh, V. Gunasekaran, *Asian J. Chem.* **2005**, 17, 1669. (b) A. M.Loriga, G. Nuvole, Pagliehi, G. Fadda, S. Zanetti, *Eur. J. Med. Chem.* **1990**, 25, 527. (c) F. Vier, . Lehendre, C. Martin, P. Rinafard, M. W. Miocque, *J. Med. Chem.* **1990**, 25, 351; (d) N. R. Pai, K. T. Waghmode, *Der Pharma Chemica*, **2012**, 4, 622.
[7] M. Zhao, T. Kamada, A. Takeuchi, H. Nishioka, T. Kuroda, Y. Takeuchi. *Bioorg. Med. Chem. Ltt.* **2015**, 25, 5551.
[8] S. Nickel, P. Nickel, M. Hellmert, S. Ernst, R. Jewell, C. A. Pearce, G. Jones, D. Hamza, M. Kaiser, *Bioorg. Med. Chem.* **2015**, 23, 2636.
[9] M. G. Sankar, L. Mantilli, J. Bull, F. Giordanetto, J. O. Bauer, C. Strohmann, H. Waldmann, K. Kumar, *Bioorg. Med. Chem.* **2015**, 23, 2614.
[10] A. Kamal, B.V. Subba Reddy, B. Sridevi, A. Ravikumar, A. Venkateswarlu, G. Sravanthi, J. P. Sridevi, P. Yogeeswari, D. Sriram, *Bioorg. Med. Chem. Lett.* **2015**, *25, 3867.*
[11] (a) I. A. Schepetkin, L. N. Kirpotina, A. I. Khlebnikov, T. S. Hanks, I. Kochetkova, D. W. Pascual, M. A. Jutila, M. T. Quinn, *Mol Pharmacol*, **2012**, 81, 832; (b) M. S. Khan, M. A. Munawar, M. Ashraf, U. Alam, A. Ata, A. M. Asiri, S. Kousar, M. A. Khan, *Bioorg. Med. Chem.* **2014**, 22, 1195.
[12] N. Nami, M. Hosseinzadeh, N. Nami, M. Haghdadi, *Phosphorus, Sulfur*, **2009**, 184, 2846.
[13] B. Tan Naional Advisory Committee for Laboratory Animal Research **2004.**
[14] L. Nargund, G. Reddy, V. Hariprasad, *J. pharm. Sc.* **1994**, 83, 246
[15] (a) A. M. Clark, F. S. El-Feraly and W.-S. Li, J. Pharm. Sci., **1981**, 70, 951. (b) A. C. Scott, in: J. G. Collee, J. P. Duguid, A. G. Fraser, B. P. Marmion (Eds.), Mackie & McCartney, "Practical Medical Microbiology", Churchill Livingstone, Edinburgh, **1989**, 161.
[16] A. Felten, B. Grandry, P. H. Lagrange, I. Casin, J. Clin. Microbiol. **2002**, 40, 2766.
[17] W. Hewitt (Eds.), "Microbiological Assay An Introduction to Quantitative Principles and Evaluation", Academic Press, New York, **1977**.
[18] Z. Kuodis, A. Rutavi˘cius, A. Matijo˘ska, O. Eicher–Lorka, *Cent. Eur. J. Chem.* **2007**, 5, 996.

[19] G. Palla, G. Predieri and P. Domiano, *Tetrahedron*, **1986**, 42, 3649.
[20] C. h. Cordier, E. Vauthier, A. Adenier, Y. Lu, A. Massat, A. Cosse–Barbi, *Struct. Chem.*, **2004**, 15, 295.
[21] A. Rutavichyus, S. Valyulene, Z. Kuodis, Chem. Heterocycl. Compd., **1997**, 33, 118.
[22] A. Rutavichyus, S. Valyulene, *Chem. Heterocycl. Compd*., **1998**, 34, 1436.
[23] A. Rutavicius, S. Valiulene and Z. Kuodis, *Chem. Heterocycl. Compd*., **1995**, 31, 629.