DR AHMED BARI (Orcid ID : 0000-0002-9447-2361)

Article type : Research Article

Title: Synthesis and anti-inflammatory activity of diversified heterocyclic systems

Running Title: Heterocyclic systems as potential drug candidates

List of Authors:

Ahmed Bari[1,2*], Daniel Grenier[3], Jabrane Azelmat[3] Saeed Ali Syed[1,2], Abdulrahman M. Al-Obaid[1], Eric C. Hosten[4]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box: 2457, Riyadh, 11451, Saudi Arabia.

*abari@ksu.edu.sa

Tel: +966533977946, Fax: +96614676220

[2]Research Center, College of Pharmacy, King Saud University, P.O. Box: 2457, Riyadh, 11451, Saudi Arabia.

[3]Oral Ecology Research Group, Faculty of Dentistry, Université Laval, Quebec City, QC, Canada, G1V 0A6.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.13576

[4]Nelson Mandela Metropolitan University, Department of Chemistry, PO Box 77000, Port Elizabeth 6031, South Africa.

Corresponding Author:

Ahmed Bari, Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box: 2457, Riyadh, 11451, Saudi Arabia.

*abari@ksu.edu.sa

Tel: +966533977946, Fax: +96614676220

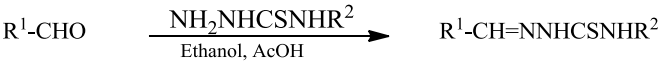
Abstract:

In continuation with our research program on the development of novel bioactive molecules, we report herein the design and synthesis of a series of diversified heterocycles (**4**-**22**). The synthesized compounds were evaluated for their anti-inflammatory activity. The chemical structures of the newly synthesized compounds have been confirmed by NMR, FTIR and micro analysis.

1. Introduction:

The prevalence of inflammatory diseases has increased rapidly over the past decades and affects more than 6 percent of world population. Although public awareness about the cause and treatment of inflammation is still growing, it remains a major health concern worldwide. Inflammatory disease, often associated with pain, fever and limited mobility, may lead to depression, hence affect the quality of life (Beekman et al., 2013). Many naturally occurring and synthetic non-steroidal anti-inflammatory drugs are based on heterocyclic core structure. These drugs are widely used to reduce pain and inflammation but given their troublesome side effects, such as nephrotoxicity and gastritis,

they are not suitable for everyone (Sharma et al., 2005).. Therefore, there is a need to develop more potent and selective. Moreover, the limited efficacy of the current therapies for inflammatory disorders supports the need for the development of alternative with less toxicity and more potency.



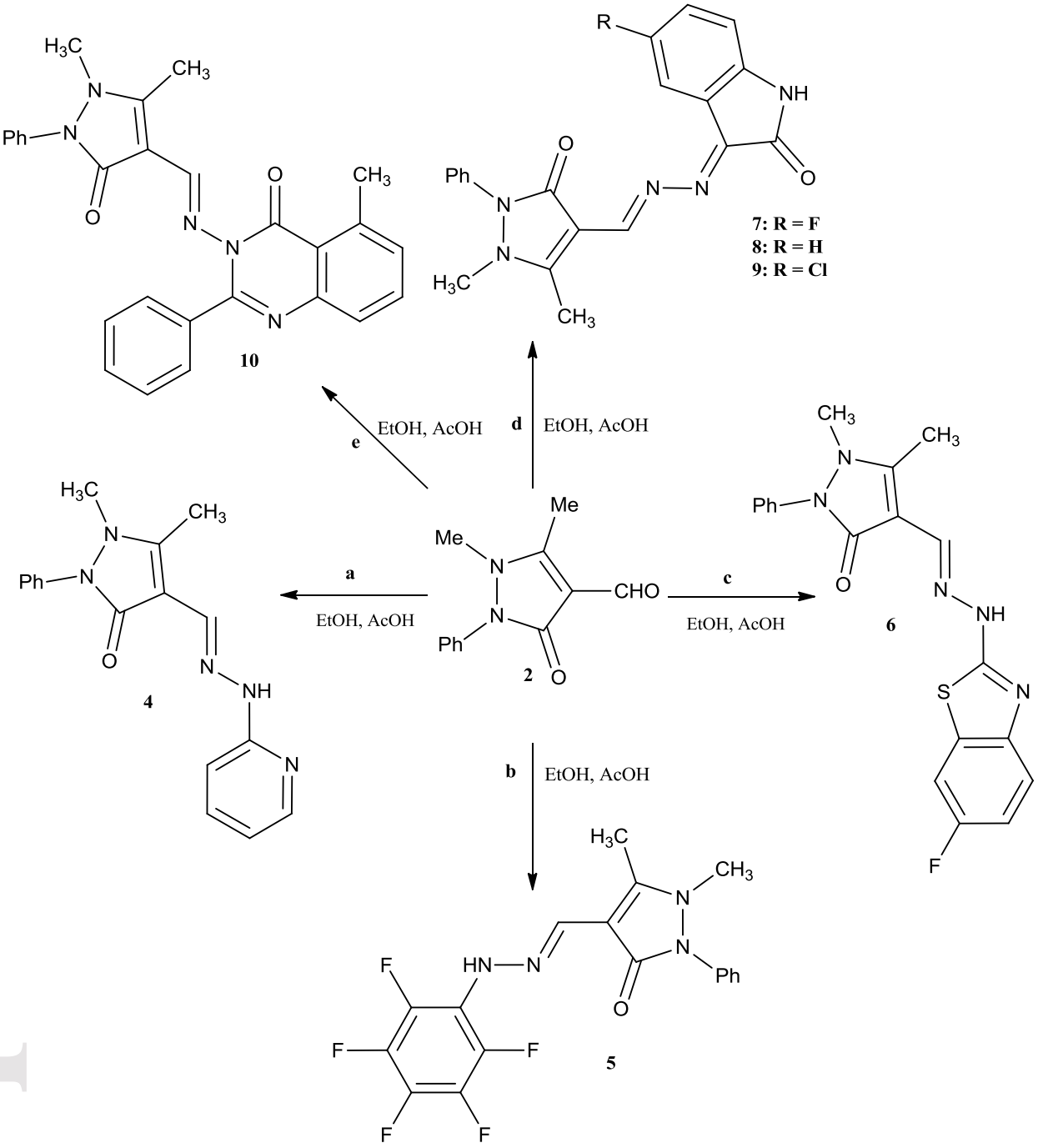
2a: R^1 = Phenazone, R^2 = H
3a: R^1 = Pyridine, R^2 = H
2b: R^1 = Phenazone, R^2 = Ph
3b: R^1 = Pyridine, R^2 = Ph

Scheme 1: Synthesis of starting materials

Despite the progress made on non-streoidal anti-inflammatory drugs, the development of new therapy methods which have both pharmacological profile and therapeutic safety represents a major concern for many scientists. Heterocyclic chemistry has generated intensive interest due to their pharmacological properties. Over the last years, several reports on the synthesis of heterocyclic molecules with anti-inflammatory potential have been published (Muralidharan et al., 2018; Fernandes et al., 2017; Rani et al., 2004). Therefore, there is a need to identify the core moiety responsible for such effects. The current paper describes the synthesis of various heterocyclic systems along with their anti-inflammatory activity in view to identify the potent nucleus. Phenazone is one of the most commonly used non-steroidal anti-inflammatory drugs (NSAID) known as cyclooxygenase and lipoxygenase inhibitors (Herxheimer et al., 1961). Formyl group attached to the heterocyclic system can also serve as a versatile tool to synthesize various biologically active analogues. Published data have revealed that the conversion of various aromatic or heterocyclic aldehyde groups into pyrazolo[*b*]quinolines is of considerable interest and often increases bioactivity both *in vivo* and *in vitro*. Furthermore, hydrazides also constitute an important class of heterocycles associated with a variety of biological properties, including antimicrobial and anticancer activities. Thiourea derivatives can be used as an intermediate for various therapeutic drugs and can also be used as an intermediate to synthesize heterocycles. Interestingly, several papers in the literature

have been published on the structural modification of thiourea (Bari et al., 2014; Fattah et al., 2010; Quiroga et al., 2001).

With the objective to develop new therapeutic agents with better anti-inflammatory activity and less toxicity, we focused our efforts to synthesize thiazolidinone derivatives. Due to the low solubility of thiosemicarbazone moiety, there are certain limitations that can be improved pharmaceutically by converting thiourea group with thiazolidinone ring which is well known for anti-inflammatory activity. There are several several reports on the synthesis of thiazole heterocycles with a protected C-4 group of the ring, however, due to the high cytotoxicity associated with the molecule, we decided to synthesize thiazolidinone moiety, which is slightly



Scheme 2: Synthesis of heterocycles from phenazone carboxaldehyde
a: 2-hydrazinopyridine, **b**: perfluoro phenylhydrazine, **c**: 2-hydrazinobenzothiazole
d: 3-hydrazineisatin, **e**: 3-aminophenyl-quinazolinone

different from thiazole, due to the presence of carbonyl group (Singh et al., 2008) at C-4.

Typically, the indolinone nucleus is a well known natural product and is extensively used as key intermediate in organic synthesis. Moreover, quinazoline-4-one is a fused heterocyclic compound and its derivatives have been extensively studied by researchers for a wide range of biological activities. In the current investigation, 1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde **2** and pyridine-3-carboxaldehyde **3** were used as starting materials to synthesize indolin-2-one and quinazoline-4-one derivatives which are often associated with antitubercular, anticancer and anti-inflammatory properties and which were previously (Kumar & Rajput, 2009) reported.

Fig. 1: Crystal structure of compound 5

2.0 Methods and Materials:

2.1 Biology:

2.1.1 **Effects of compounds on cell viability**

Prior to evaluating the anti-inflammatory properties of the compounds, we determined their cytotoxic effect on the U937 3xκB-LUC cell line to exclude the possibility that toxicity related to the compounds might cause a decrease in NF-κB activation. The cells were treated for 6.5 h with two-fold serial dilutions of the compounds, starting at 1000 µM. Thereafter, a colorimetric MTT cell viability assay (Roche Diagnostics, Germany) using 3-[4,5-diethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide as the substrate was performed according to the manufacturer's protocol. Untreated control cells were assigned a value of 100%.

2.1.2 **Activation of the NF-κB transcription factor**

The human monoblastic leukemia cell line U937 3xκB-LUC, a subclone of the U937 cell line stably transfected with a luciferase gene coupled to a promoter of three NF-κB-binding sites, was used to

investigate the effect of the compounds on lipopolysaccharide (LPS)-induced NF-κB activation. Cells were routinely cultivated in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 µg/ml of penicillin G/streptomycin, and 75 µg/ml of hygromycin B at 37°C in a 5% CO_2 atmosphere. To induce activation of the NF-κB transcription factor, the U937 3xκB-LUC cells (10^6 cells/well) were placed into wells of a black wall, black bottom 96-well microplate (Greiner Bio-One North America Inc.) and were stimulated for 6 h with *Aggregatibacter actinomycetemcomitans* ATCC 20522 LPS (1 µg/ml). Thereafter, to investigate the effect of the compounds on LPS-induced NF-κB activation, U937 3xκB-LUC cells were pre-incubated with non-cytotoxic concentrations of the compounds (in RPMI containing 1% FBS) for 30 min and were then stimulated for 6 h with LPS. Wells with no LPS or no compounds were used as controls to measure basal NF-κB activity. An assay using the commercial inhibitor BAY-11-7082 (25 µM) was used as a positive control for inhibition of NF-κB signaling pathway. NF-κB activation was quantify by measuring luciferase activity following the addition Bright-Glo™ reagent (Promega Corporation, Madison, WI, USA) in accordance with manufacturer's protocol. Luminescence was monitored using a Synergy 2 microplate reader.

Fig. 4: Crystal structure of compound 22

2.2 Chemistry:

All solvents and reagents were purchased from Aldrich Chemical Co and were used as obtained. The reactions were carried out under air atmosphere unless stated otherwise. Melting points measured with a Thermo Scientific 9100 apparatus and are uncorrected. IR spectra were recorded with a Bruker Alpha spectrometer using ATR accessory. ^{1}H- and ^{13}C-NMR spectrum was recorded on a Bruker instrument at 500 and 700 MHz for ^{1}H and 125 MHz for ^{13}C at 295 K in DMSO-d_6 and $CDCl_3$. The mass spectrometric experiments were carried out on a Jeol JMS-700 mass spectrometer. Thin-layer chromatography (TLC) was performed on a fluorescent aluminium backed silica gel HF^{254} plates

(Merck) and was viewed under UV 254 and 265 lights and charring with EtOH/H_2SO_4. Micro analysis was carried out on a Perkin Elmer CHNS 2400 instrument. Merck silica gel 60 (230-400 mesh) was used for column chromatography separations.

2.2.1 **General procedure for the synthesis of 2a, 2b and 3a, 3b:**

To a solution of pyridine-3-carboxaldehyde or phenazone-4-carboxaldehyde (1 mmol) in absolute ethanol was added respective thiosemicarbazide (1.2 mmol) followed by few drops of acetic acid. The reaction mixture was refluxed with stirring for 3 hrs followed by the addition of ice. The solid separated was collected by filtration and washed several times with water.

2.2.2 **General procedure for the synthesis of compound 5-10, 16, 17:**

To a solution of aldehyde **1** (2 mmol) in absolute ethanol (50 mL) was added respective hydrazine (2.2 mmol) and catalytic amount of acetic acid. The resulting mixture was refluxed for 3 h with stirring followed by the evaporation of solvent in vacuo. The solid obtained was washed with cold water several times and recrystallized with ethanol.

2.2.3 **General procedure for the synthesis of compound 11:**

To a mixture of thiourea derivatives **2a** (2 mmol) in acetonitrile (30 mL) was added maleic anhydride slowly in 10 mins time at room temperature. The reaction mixture was than refluxed for 5 h with stirring followed by the addition of ice. The obtained precipitate was collected by filtration, washed with water and recrystallized with ethanol afforded cyclized product in good yield.

2.2.4 **General procedure for the synthesis of compound 12, 13, 20:**

To a solution of thiourea derivatives **2a**, **3a or 2b** (2 mmol) in glacial acetic acid was added anhydrous sodium acetate (2.2 mmol) and monochloroacetic acid (2.2 mmol). The mixture was refluxed for 4 h with stirring followed by the addition of ice. The obtained precipitate was collected by filtration, washed with water and recrystallized with cold ethanol.

***2.2.5* General procedure for the synthesis of compound 18, 19:**

A mixture of thiourea derivatives **2a** (2 mmol) in aceticanhydride (30 mL) was refluxed for 5 h with stirring followed by the addition of ice. The obtained precipitate was collected by filtration, washed with water and recrystallized with ethanol afforded cyclized products in good yields.

***2.2.6* General procedure for the synthesis of compound 15:**

To a solution of aldehyde **2** (2 mmol) in absolute ethanol (50 mL) was added dimedone (4.2 mmol) and ammoniumacetate (2.2 mmol). The resulting mixture was stirred at room temperature for 10 min than PPA/SiO_2 (50 mg) was added. The reaction mixture was refluxed for 4 h, filtered and evaporation of solvent in vacuo resulted in the precipitate. The solid obtained was washed with cold water several times afforded compounds **2** and **3**.

***2.2.7* General procedure for the synthesis of compound 14, 21, 22:**

To a solution of aldehyde **1** (2 mmol) in absolute ethanol (50 mL) was added dimedone (3 mmol), ammoniumacetate (2.2 mmol) and appropriate amine (2.2 mmol). The resulting mixture was cooled in an ice bath and sodium metal (2.2 mmol) in ethanol was added dropwise. After complete addition, the mixture was refluxed for 4 h, filtered and evaporation of solvent in vacuo

resulted in the precipitate. The solid obtained was washed with cold water several times, dried and purified by column chromatography (1:1 EtOAc-Hexane) afforded compounds **2** and **3**.

Fig. 5: NF-$_k$B inhibition and cytotoxicity data

Fig. 6: NF-$_k$B inhibition and cytotoxicity data

2.3 X-ray Crystallographic Data: Crystals of compound **45, 13, 14a and 22** were obtained by allowing slow solvent evaporation. Crystallographic data have been deposited at the Cambridge Crystallographic Data Center (CCDC Nr for compound **5** is1896597, CCDC

Nr for compound **13** is 1896596, CCDC Nr for compound **14a** is 1896365 and CCDC Nr for compound **22** is 1896364).

3 Results and Discussion:

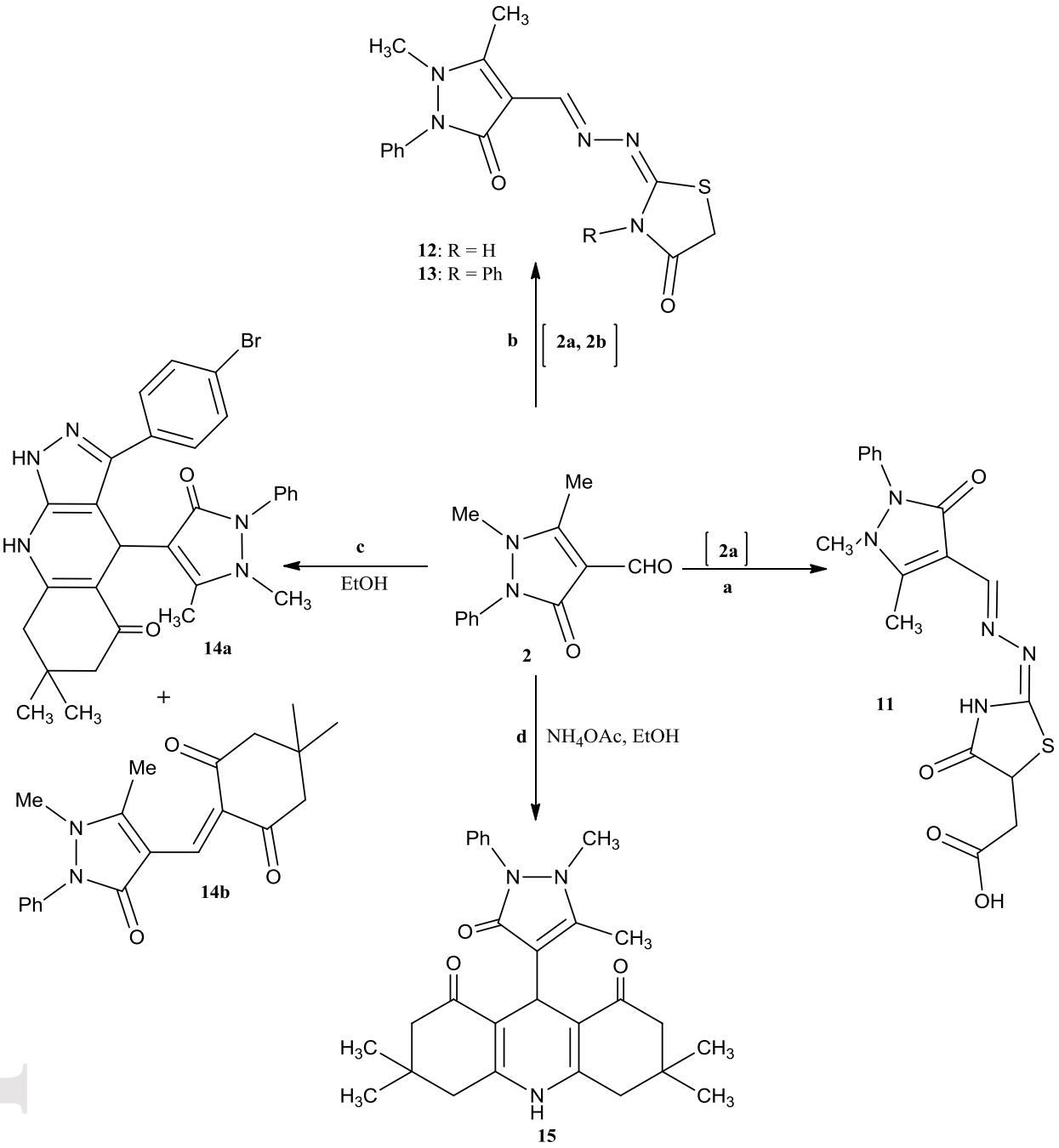
3.1 Chemistry:

For the synthesis of compounds **2a and 3a**, 1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carbaldehyde **2** and pyridine-3-carboxaldehyde **3** were treated with chosen thiosemicarbazides in refluxing ethanol with few drops of acetic acid. The precipitate obtained was washed several times with water and used without further purification (**scheme 1**).

Reaction of carboxaldehydes **2** and **3** with various substituted hydrazines afforded schiff bases **4-10**, **16** and **17** in acidic medium. Reaction with various isatin derivatives are of utmost importance because indoline-2-one molecules are well known for their antimicrobial, antiviral and anticancer properties. Disappearance of formyl proton and a presence of a singlet at around 8 ppm for CH and a broad peak in the downfield region for NH confirm the postulated structure. Moreover, IR and mass spectra are also in accordance with the structures. The compound **5** was crystallized in ethanol afforded crystals suitable for x-ray crystallography (**fig 1**).

In the current paper, construction of thiazolidine heterocyclic system was carried out by reaction of substituted thiosemicarbazone with monochloroacetic acid and maleicanhydride in acetic acid affording **11**, **12**, **13** and **20** in excellent yields (scheme **3** and **4**). More constrained thiazolidine ring system was selected due to their involvement in molecules reported as anti-inflammatory (Gupta, Sundaram & Reuter, 2010).

Fig. 2: Crystal structure of compound 13

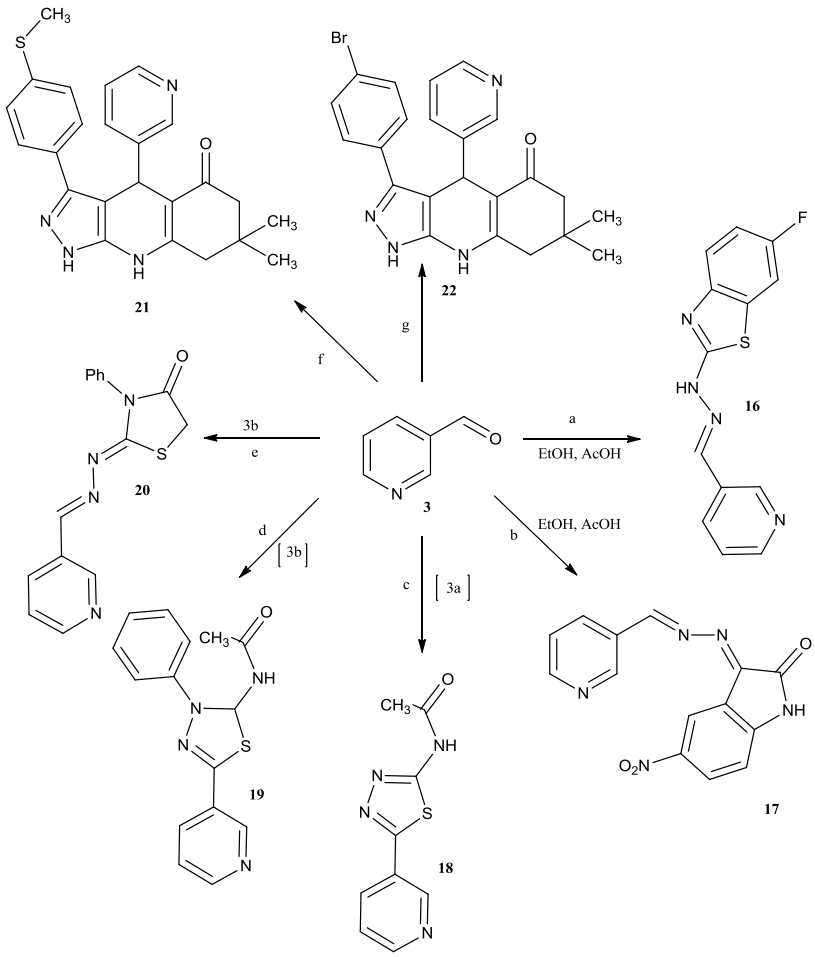


Scheme 3: Synthesis of thiazolidine and quinazoline type heterocyclic systems
a: maleicanhydride, CH_3CN **b**: chloroacetic acid, sodiumacetate, AcOH
c: 5-amino-3(4-bromophenyl)pyrazole, dimedone, PPA/SiO_2 **d**: dimedone,PPA/SiO_2

Structure of compound **13** was also confirmed by x-ray crystallography which was obtained by slow evaporation of ethanol (**fig 2**). On the basis of reaction time and the corresponding yields that the

intermediate thiourea derivatives with phenyl group is less reactive than the other one because of the steric effect of phenyl group that reduces the ability of NH undergo nucleophilic cyclization. Same was observed when the intermediate (NH) was reacted with acetic anhydride **18** and **19**. The NH_2 due to strong nucleophilicity may facilitate substitution with acetic anhydride. However, the resonance effect between NH and the phenyl group of intermediate reduces the nucleophilic substitution with the steric effect of phenyl group further plays a role in reducing yields (Muscia & Buldain, 2014). The reaction of **2a** and **2b** with acetic anhydride was unsuccessful even after prolonged reaction time and reflux. Appearance of CH of the thiazolidine ring at around 4 ppm and NH in the downfield region (11 to 12 ppm) confirms the ring transformation reaction. Mass, FTIR and elemental analysis also confirm the postulated structures. All the synthesized compounds were washed with water and cold ethanol prior to characterization by spectroscopic method and elemental analysis.

In the synthesis of quinazoline-4-one (**10**) type of molecules, more time was required due to the steric hindrance caused by the phenyl group making the amino group less available for cyclization. However, the quinazoline-5-one ring system which is of considerable importance due to their biological activity was also synthesized. Several reports for the synthesis of such systems involved Friedlander approach but due to the formation of various side products there is a need to develop a more efficient approach with higher yields (Kumar, Kaur & Kumari, 2012). Therefore, for the synthesis of quinazoline-5-one heterocyclic system **14a**, **15**, **21** and **22**, silica gel supported polyphosphoric acid (PPA-SiO_2) as a green and reusable heterogeneous catalyst was utilized. In the course of the



Scheme 4: Synthesis of diversified heterocyclic systems from pyridine-3-carboxaldehyde
a:2-hydrazinobenzothiazole **b**: 5-nitro-3-hydrazineisatin **c**: aceticanhydride **d**: aceticanhydride **e**: chloroaceticacid, sodiumacetate, AcOH **f**: 3(4-methylthio)phenylpyrazole-5-amine, dimedone,PPA/SiO_2 **g**: 5-amino-3(4-bromophenyl)pyrazole, dimedone, PPA/SiO_2

synthesis of **14a**, cyclohexane-1,3-dione **14b** was obtained as by product which is assumed to be due to the Knoevenageal condensation which is a initial step in the cyclocondensation reaction. The NMR spectroscopic data is also consistent with the proposed structures, for example compounds **14a**, **21** and **22**, exhibit in a ^{1}H NMR spectrum, the two relatively sharp singlets which are readily assigned as exchangeable NH-groups. Moreover another two couples of doublets appear around 2-3 ppm corresponding to the diastereoscopic CH_2 groups respectively. Proposed molecule is further confirmed by x-ray crystallographic studies of compound **14a** (**fig 3**) and **22** (fig **4**) which were obtained in ethanol-dimethylsulfoxide mixture. However, when **2** were treated with two equivalents

of dimedone, in the presence of ammonium acetate, compound **15** was obtained in high yield. The structures were assigned using ^{13}C, DEPT, HMBC and HSQC experiments.

Fig. 3: Crystal structure of compound 14a

3.2 Biology:

In the present study, we used the U937-3xκB-LUC human monocytic cell line, which contains a luciferase reporter possessing three NF- κB binding sites, to evaluate the ability of the designed molecules to inhibit activation of the NF-κB signaling pathway. This model is very convenient to rapidly evaluate the potential of new compounds to block activation of the transcription factor NF-κB, an important regulator of many pro-inflammatory pathways (Cardinal et al., 2017; Lagha & Grenier, 2016; Lagha, LeBel & Grenier, 2018). First, to exclude the possibility that cell toxicity might have been responsible for any decrease in NF-κB activation, the viability of the U937-3xκB-LUC cells treated with the molecules was evaluated using an MTT test. **Table 1** reports the highest concentration of the tested molecules for which no significant toxicity was found. On the one hand, some molecules were found to be cytotoxic at low concentrations; for instance the highest non-cytotoxic concentration for **16** was 0.24 µM while for **6**, **19**, and **22** was 1.95 µM. On the other hand, some other molecules were much less cytotoxic; for instance the highest non-cytotoxic concentration for **2a**, **3a**, **7**, and **17** was 500 µM while for **11**, and **18** was 250 µM.

Two-fold serial dilutions of the highest non-cytotoxic concentration of each molecule were then tested for their ability to inhibit the LPS-induced activation of NF-κB signaling pathway (**Fig 5, 6**). While some molecules did not show any inhibition (**4**, **9**, **12**, **16**, **19**, **20**, and **21**), others significantly and dose-dependently inhibited LPS-induced NF-κB activation. The most effective molecules were **2a**, **3a**, **6**, **11**, and **15** and their IC_{50} values calculated were as below:

2a : 183.7 ± 4.6 µM

3a : 115.4 ± 4.6 μM

6 : 0.5 ± 0.2 μM

11 : 107.4 ± 7.6 μM

15 : 43.3 ± 12.9 μM

Given that the transcription factor NF-κB controls the expression of a large array of genes involved in inflammation, some of the novel molecules by inhibiting NF-κB activation shows potential to reduce the host inflammatory response.

Thiosemicarbazide of both starting materials **2a** and **3a** bearing a free NH_2 group showed excellent inhibition as compared to the phenylthiosemicarbazide derivatives **2b** and **3b**. This is assumed to be due to the steric hindrince caused by the phenyl group which plays a major role in reducing cytotoxicity.

The schiff base of phenazone carboxaldehyde **6** enhances inhibition in comparision with benzothiazole schiff base of pyridine **16**. This could be due to the biological activity associated with phenazone moeity itself while the benzothiazole bearing a fluoro group also plays a role in increasing biological activity. The cyclization of thiosemicarbazono group to the oxathiozolidine bearing exocyclic aceticacid **11** also showed excellent inhibition. It was anticipated that the presence of the elongated substituent in the thiazolidine ring at C-5 enhance the inhibition considerably. Moreover, the impact of phenazone group in the overall activity is also taken in account. Hexahydroacridine **15** having phenazone group at C-9 also showed marked inhibition as compared to other analogues suggesting that the two diketone groups attached to acridine nucleus seems more

favourable for constructing an antiinflammatory agent than the case of substitution with electron withdrawing group.

Conclusion:

In conclusion, the newly synthesized compounds having phenazone group were found to be more potent than the corresponding pyridine analogues. But, additional studies are required to investigate the stability of hydrazones such as **11** in biological systems.

Acknowledgement:

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no RG-1440-009.

Conflict of Interest:

The authors declare no competing financial interest.

Supplementary Information:

Supplementary information (spectroscopic data, ^{1}H NMR and Mass spectra) is available free of charge as PDF file.

4.0 References:

Beekman, A. T., Jonge, De. P & Penninx, B. H. (2013), Anxiety disorders and inflammation in a large adult cohort. ***Translational Psychiatry***. *3*, 249-253. 10.1038/tp.2013.27.

Bari, A., Hashmi, I. A., Syed, S. A. (2014). Synthesis of Some New Heterocyclic Compounds Derived from 3-Formylchromones and Their Antimicrobial Evaluation, ***Chemistry of Heterocyclic Compounds***, 49, *12*, 1723-1726. 10.1007/s10593-014-1424-4

Cardinal, S., Alexandre, P., Cote, P., Azelmat, J., Bouchard, C., Grenier, D. (2017). Synthesis and anti-inflammatory activity of isoquebecol. ***Bioorganic& Medicinal Chemistry***, *25*, 7, 2043. 10.1016/j.bmc.2017.01.050. Epub

Fattah, F. A., Nehad, R & El-Sayed, A. (2010). Synthesis and anticonvulsant activity of certain substituted furochromone, benzofuran and flavone derivatives. ***Chem. Pharm. Bull***, *58*, 9, 1148-1156. https://doi.org/10.1248/cpb.58.1148.

Fernandes, J., Revanasiddappa, B. C., Ishwarbhat, K., Kumar, M. V., D'Souza, L., & Alva, S. (2017). Synthesis and in-vitro Anti-Inflammatory Activity of Novel Pyrazoline Derivatives. ***Res. J. Pharm. Technol***, *10*, 1679-1682. https://doi.org/10.22159/ijpps.2018v10i2.23772.

Gupta, S. C., Sundaram, C., Reuter S. (2010). Inhibiting NF-κB activation by small molecules as a therapeutic strategy. ***Biochim. Biophys. Acta***, *1799*, 10-12, 775.

doi: 10.1016/j.bbagrm.2010.05.004

Herxheimer, H & Stresemann, E. (1961). Anti-asthmatic effect of phenazone and amidopyrine, ***Nature****, 192*, 1089. doi.org/10.1038/1921089b0.

Kumar, A & Rajput C, S. (2009). Synthesis and anti-inflammatory activity of newer quinazolin
4-one derivatives. ***Eur. J. Med. Chem***, *44*, 1, 83. 10.1016/j.ejmech.2008.03.018.

Kumar, R., Kaur, M., Kumari, M. (2012). Acridine: a versatile heterocyclic nucleus. ***Acta Poloniae Pharmaceutica*: *Drug Research***, *69*, 1, 349. http://ptfarm.pl/pub/File/Acta_Poloniae/2012/1/003.pdf

Lagha, B., LeBel, G., Grenier D. (2018). Dual action of highbush blueberry proanthocyanidins on Aggregatibacter actinomycetemcomitans and the host inflammatory response. ***BMC Complementary and Alternative Medicine,*** 2018, *18*, 10. doi: 10.1186/s12906-017-2072-x

Lagha, A. B., Grenier, D. (2016). Tea polyphenols inhibit the activation of NF-κB and the secretion of cytokines and matrix metalloproteinases by macrophages stimulated with Fusobacterium nucleatum. ***Sci Rep***, 2016, 6, 34520. 10.1038/srep34520

Muralidharan, V., Deepti, A & Raja S. (2018). *International journal of pharmacy and pharmaceutical sciences*, A review on anti-inflammatory potential of substituted pyrazoline derivatives synthesised from chalcones. 10, *2*, 9-14. *10*, 2. dx.doi.org/10.22159

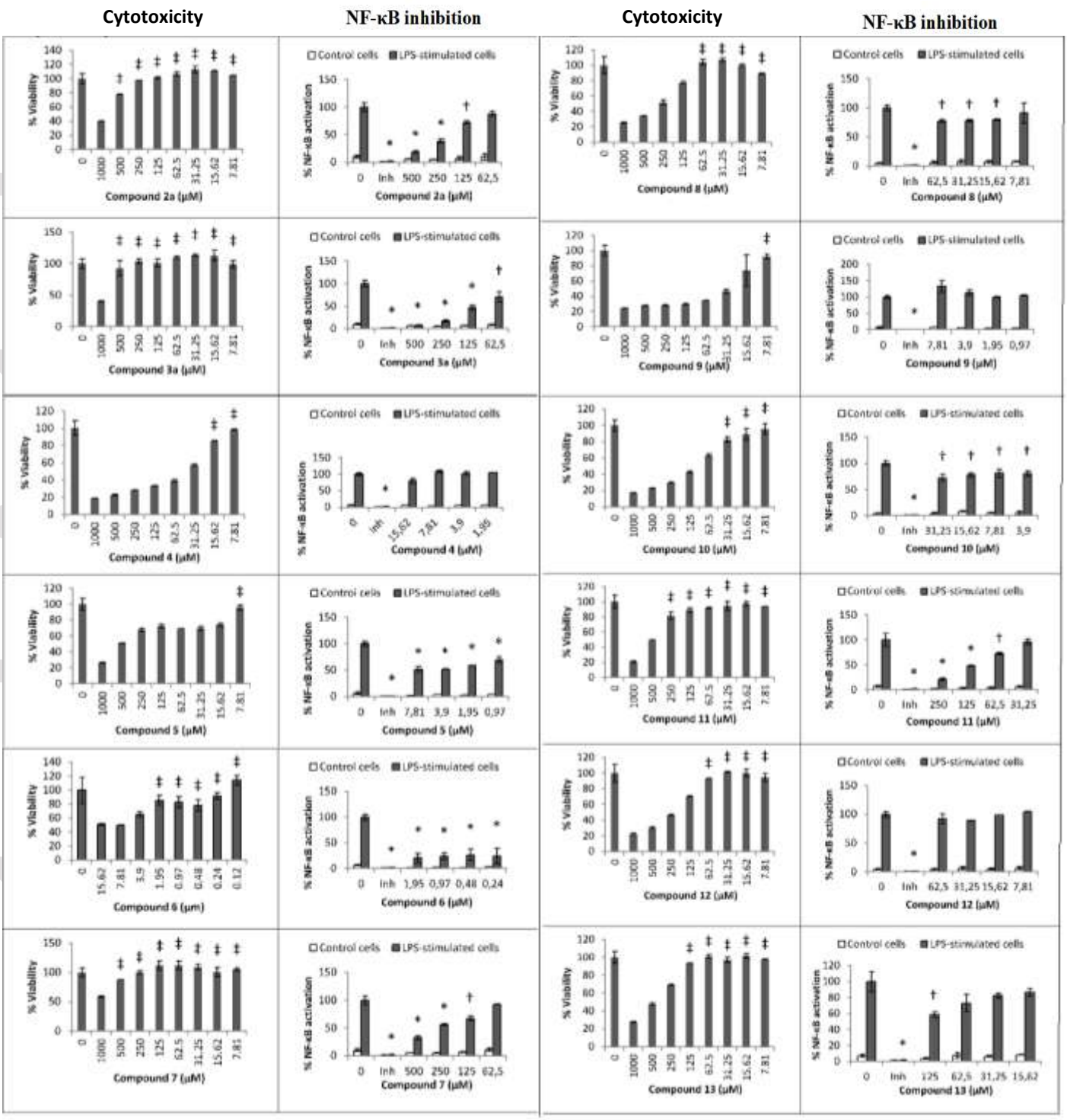
Muscia, G. C., Buldain, G. Y. (2014). Design, synthesis and evaluation of acridine and fused-quinoline derivatives as potential anti-tuberculosis agents. ***Eur. J. Med. Chem***, *12*, 73, 243. 10.1016/j.ejmech.2013.12.013

Quiroga, J., Mejia, D., Insuasty, B & Abonia, R. (2001). Regioselective synthesis of 4, 7, 8, 9-tetrahydro-2H-pyrazolo[3, 4-b] quinolin-5 (6H)-ones. Mechanism and structural analysis, ***Tetrahedron***, 2001, *57*, 6947. doi.org/10.1016/S0040-4020(01)00649-4

Rajput, C. S., & Kumar, A. (2009). Synthesis and anti-inflammatory activity of newer quinazolin-4-one derivatives. ***Eur. J. Med. Chem***, *44* (1), 83, 10-15. 1016/j.ejmech.2008.03.018

Rani, P., Srinavastava, V. K & Kumar (2004). A. Synthesis and antiinflammatory activity of heterocyclic indole derivatives, ***Eur. J. Med. Chem***, 2004, *39*, 5, 449-452. 10.1016/j.ejmech.2003.11.002.

Singh, N., Bhati, S. K & Kumar, A. (2008). Thiazolyl/oxazolyl formazanyl indoles as potent anti-inflammatory agents. ***Eur. J. Med. Chem***. 43 (11), 2597-2603. 10.1016/j.ejmech.2007.12.024**.**



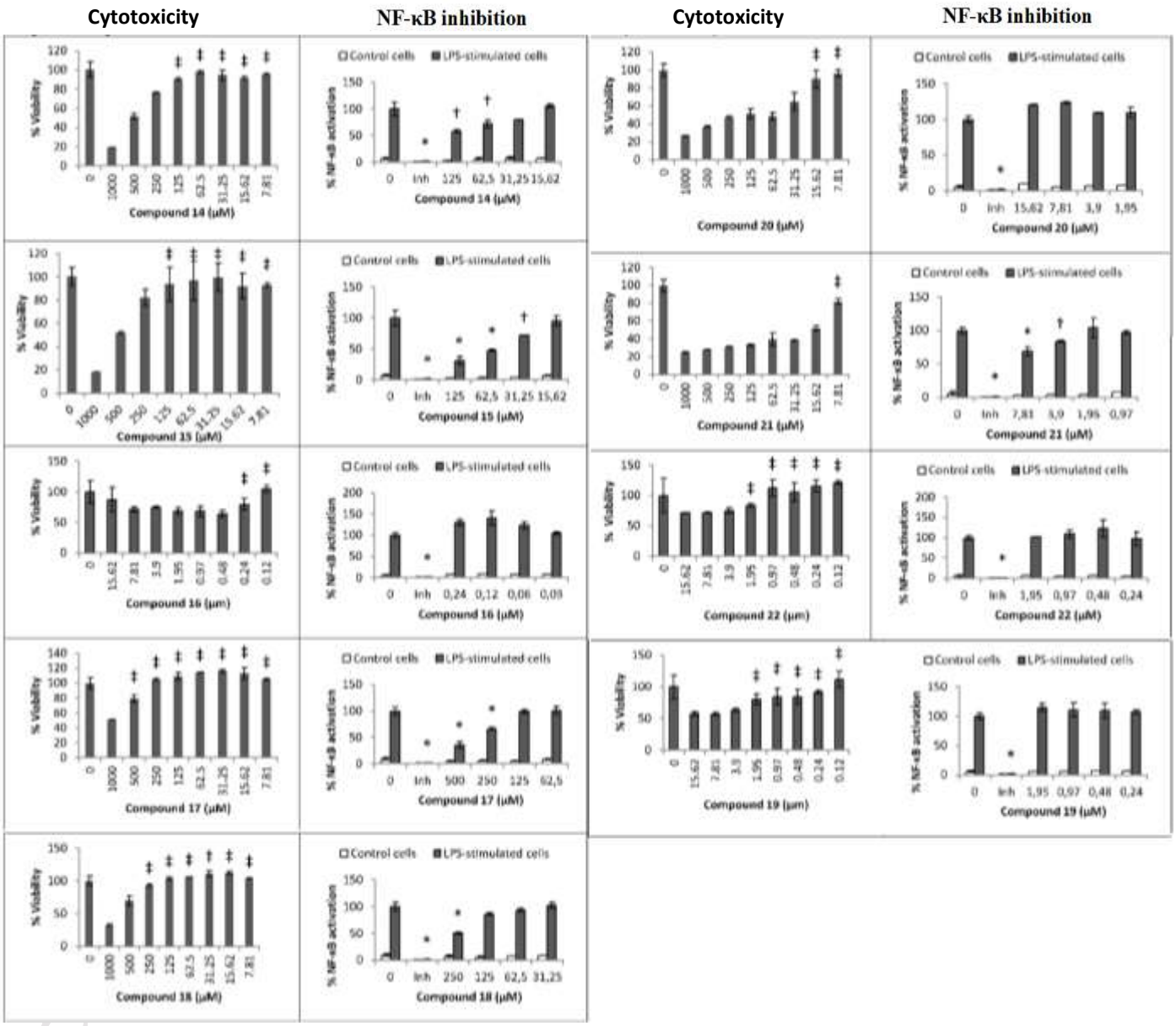
‡ : Considered as non-cytotoxic

Inh : NF-κB inhibitor (BAY 11-7082)

* : significantly different from LPS-stimulated cells at p ‹ 0.01

† : significantly different from LPS-stimulated cells at p ‹ 0.05

Fig. 5: NF-$_k$B inhibition and cytotoxicity data



‡ : Considered as non-cytotoxic

Inh : NF-κB inhibitor (BAY 11-7082)

* : significantly different from LPS-stimulated cells at $p < 0.01$

† : significantly different from LPS-stimulated cells at $p < 0.05$

Fig. 6: NF-$_k$B inhibition and cytotoxicity data

Table 1. Cytotoxicity of the molecules towards the U937-3xκB-LUC human monocytic cell line, as determined with a MTT colorimetric assay.

Molecule	Highest concentration showing no cytotoxicity (µM)
2a	500
3a	500
4	15.62
5	7.81
6	1.95
7	500
8	62.5
9	7.81
10	31.25
11	250
12	62.5
13	125
14	125
15	125
16	0.24
17	500
18	250
19	1.95
20	15.62
21	7.81
22	1.95

Fig. 1: Crystal structure of compound 5

Fig. 2: Crystal structure of compound 13

Fig. 3: Crystal structure of compound 14a

Fig. 4: Crystal structure of compound 22

Fig. 5: NF-$_k$B inhibition and cytotoxicity data

Fig. 6: NF-$_k$B inhibition and cytotoxicity data

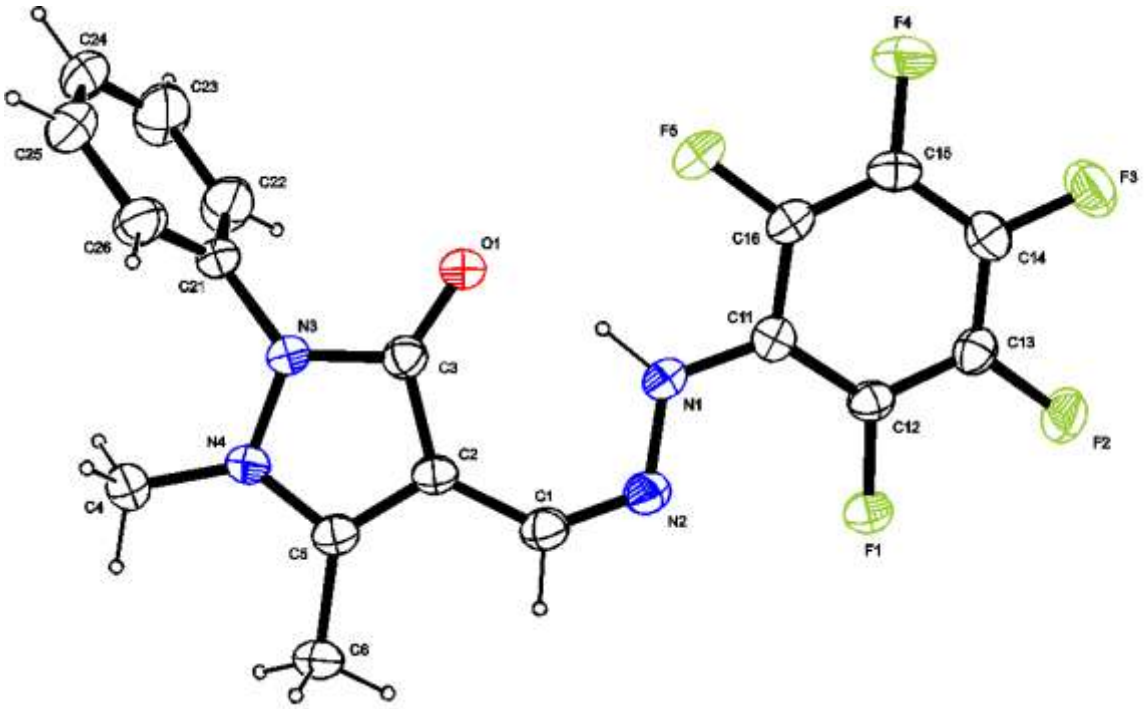


Fig 1

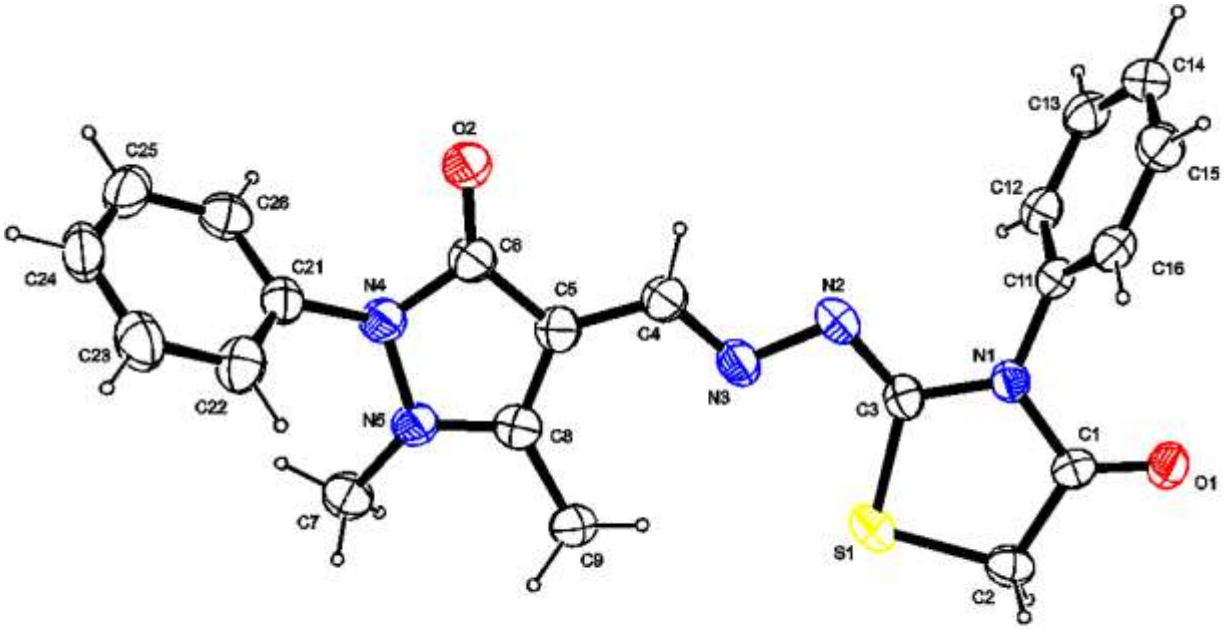


Fig 2

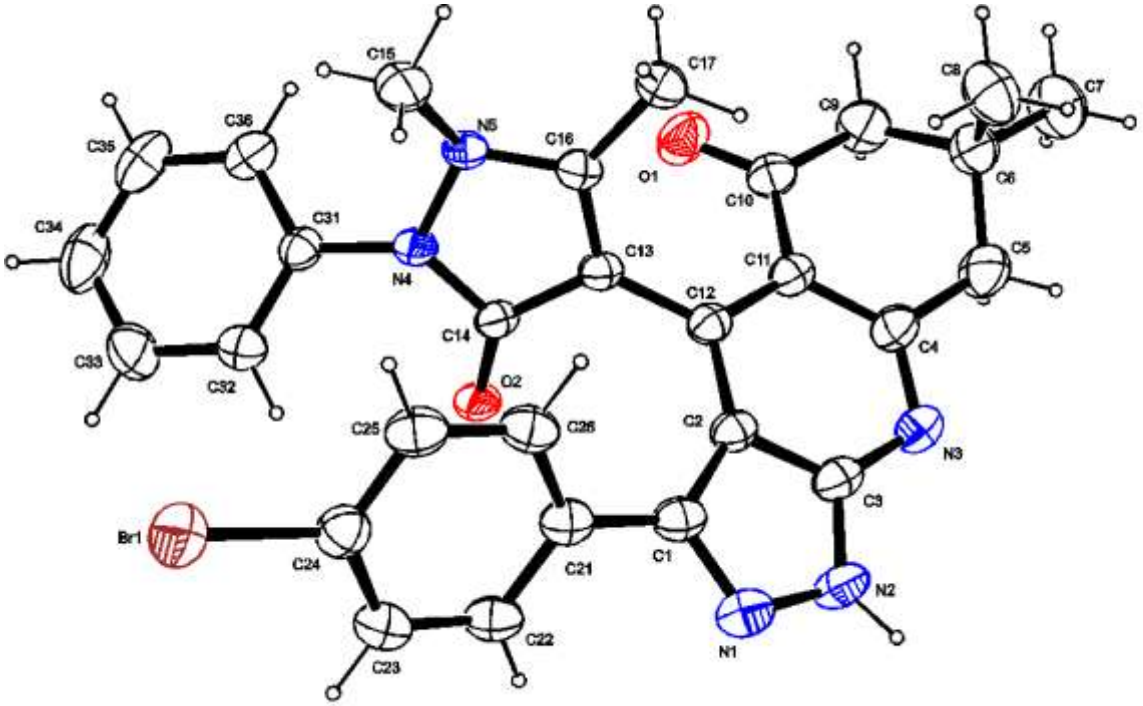


Fig 3

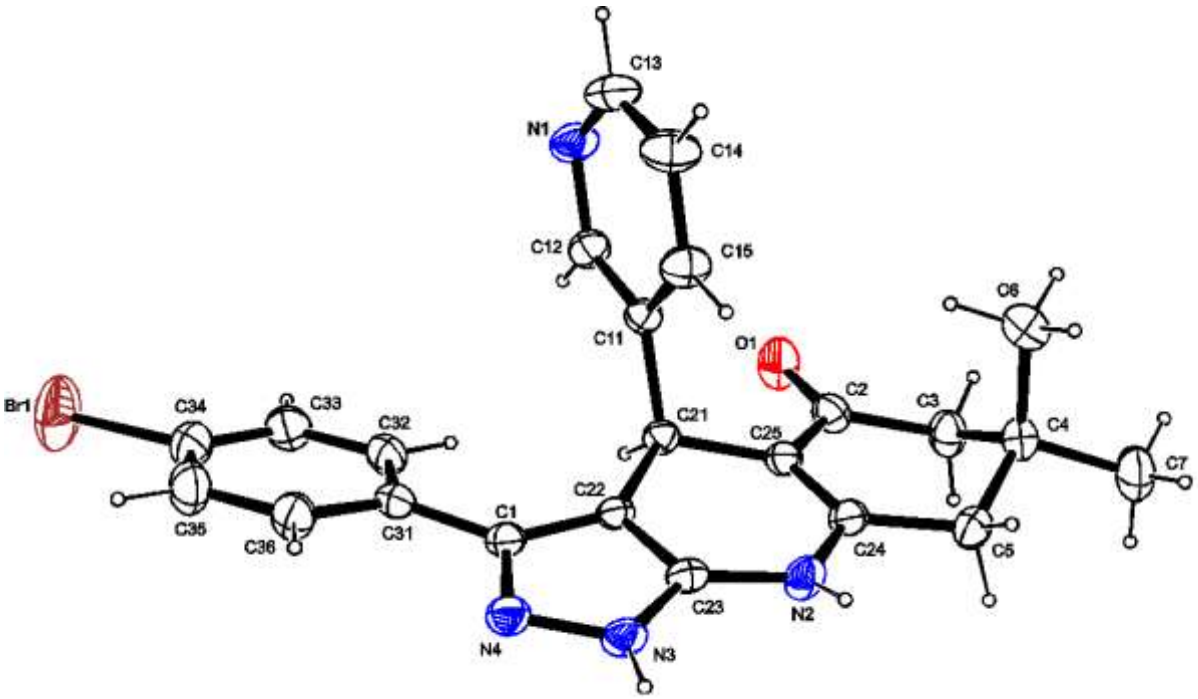


Fig 4