



Research Article

## A MINI REVIEW ON BIOLOGICAL ACTIVITIES OF 6-(4-CHLOROPHENYOXY)-TETRAZOLO [5,1-A] PHTHALAZINE (QUAN-0808) COMPOUND

Mohammad Asif

Department of Pharmacy, GRD(PG)IMT, Dehradun - 248009, (Uttarakhand), India

### Abstract:

**Purpose:** QUAN-0808 (6-(4-chlorophenoxy)-tetrazolo[5,1-a]phthalazine) was tested for the anticonvulsant, anti-inflammatory, analgesic, anticoagulant, antithrombotic and antidepressant effects.

**Approaches:** Anticonvulsant activity was tested by electroshock seizure model and neurotoxicity was tested by the rotarod neurotoxicity test in mice. In chemically induced models of seizure like pentylenetetrazole, isoniazid, thiosemicarbazide and 3-mercaptopropionic acid were further tested for the anticonvulsant activity.

**Findings:** QUAN-0808 caused significant anticonvulsant activity against all types of seizures. It appreciably reduced xylene-induced ear edema, reduced the prostaglandin E2 and nitric oxide levels on the edema and reduced acetic acid-induced capillary permeability hyperactivity and reduced acetic acid-induced writhing response. It exhibited anti-inflammatory and antinociceptive effect in a dose-dependent manner.

**Conclusions:** The peripheral effect mechanisms of QUAN-0808 may be related to a reduced in the formation of PGE2, NO, bradykinin and additional inflammatory mediators. The anticoagulant and antithrombotic effects of Q808 delayed bleeding and clotting time in mice. QUAN-0808 exerts anticoagulant, antithrombotic and antidepressant effect, exhibited a significant reduction in immobility as antidepressant.

**Key words:** *Tetrazolo-phthalazine, anticonvulsant, antidepressive, QUAN-0808, anti-inflammatory, antinociceptive, antithrombotic.*

Received on : 22-10-2016

Revised on : 28-12-2016

Accepted on : 20-03-2017

### INTRODUCTION

Compound I (6-(4-chlorophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine; was first recognized for anticonvulsant activity with an ED<sub>50</sub> value of 7.1 mg/kg against ME

S-induced seizures.<sup>1,2</sup> The anticonvulsant activity of compound I was slightly higher than that of the reference drug (Carbamazepine) in the MES test, but the adverse effects of compound I were marked with

#### Corresponding Author

Mohammad Asif

Department of Pharmacy,

GRD(PG)IMT,

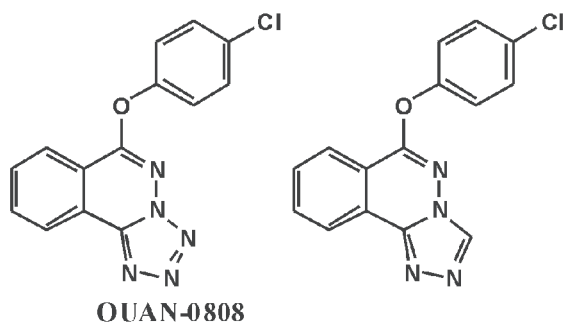
Dehradun, 248 009,

(Uttarakhand), India

E mail: aasif321@gmail.com

a TD<sub>50</sub> of 36.7 mg/kg. In our attempts to find compounds with lower neurotoxicity, we replaced the -CH= in the triazole by an N atom, according to the theory of bioisosterism and synthesized a series of tetrazolo [5,1-a]phthalazine derivatives that resulted in the compound QUAN-0808 with a 4-chlorophenoxy group substitution at position 6. The tetrazole and triazole moieties exhibit bioisosterism. The peak time of protection of QUAN-0808 after oral administration was also evaluated using the maximal electro-shock (MES) test and we compared the anticonvulsant activity of QUAN-0808 with the reference drug, carbamazepine, at this peak time. The tetrazole ring containing compounds have attracted rising attention because of their marked anti-inflammatory

and antimicrobial<sup>3</sup>, anti-hypertensive<sup>4</sup> activities and because of their inhibition of benzodiazepine receptor binding. QUAN-0808 has low toxicity ( $TD_{50} > 4500$  mg/kg in mice).<sup>5</sup> In 20 mice were given an orally used dose of 6000 mg/kg of QUAN-0808 and no death was observed over a period of seven days. QUAN-0808 is also has anticoagulant, antithrombotic effects as well as antidepressant



### ANTICONVULSANT ACTIVITY

Epilepsy, a disease characterized by recurrent seizures, affects more than 60 million people worldwide. Nearly 95% of the drugs currently available to treat epilepsy provide satisfactory seizure control in 60–70% of patients. However, these drugs are linked both with remarkable adverse side effects, like drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia and life-threatening conditions.<sup>6</sup> Therefore, the continued search for safer and more effective antiepileptic drugs (AEDs) is challenge for medicinal chemistry. The anticonvulsant activity of phthalazine tetrazole derivative, QUAN-0808 (6-(4-chlorophenoxy)-tetrazolo[5,1-a]phthalazine) by MES seizure model and neurotoxicity was tested by the rotarod neurotoxicity test in mice. QUAN-0808 exhibited higher activity (median effective dose,  $ED_{50} = 6.8$  mg/kg) and lower neurotoxicity (median toxic dose,  $TD_{50} = 456.4$  mg/kg), resulting in a higher protective index ( $PI = 67.1$ ) compared with carbamazepine ( $PI = 6.4$ ). QUAN-0808 exhibited significant oral anticonvulsant activity ( $ED_{50} = 24$  mg/kg) against MES-induced seizure with low neurotoxicity ( $TD_{50} > 4500$  mg/kg) in mice, resulting in a PI value of more than 187.5. QUAN-0808 was also tested in chemically induced models of seizure pentylenetetrazole [PTZ], isoniazid [ISO], thiosemicarbazide [THIO] and 3-mercaptopropionic acid [3-MP]) to investigate the anticonvulsant activity;

QUAN-0808 produced significant anticonvulsant activity against seizures induced by ISO, THIO and 3-MP.<sup>7,8</sup>

The anticonvulsant activities of QUAN-0808 compared with reference drug and showed a dose-dependent anticonvulsant effect with an  $ED_{50}$  of 6.8 mg/kg and a  $TD_{50}$  of 456.4 mg/kg, resulting in a PI of 67.1 in MES model. QUAN-0808 possesses not only greater anticonvulsant activity but also lower toxicity than the reference drug in the MES test. The peak effect of the oral dose of 30 mg/kg QUAN-0808 occurred between 0.25 and 6 h after administration. QUAN-0808 was evaluated for its oral activity against MES-induced seizures and oral neurotoxicity in mice, with carbamazepine as the reference drug. The time to peak effect was 2 h, which was comparable to the peak effect of carbamazepine. The increase in the anticonvulsant potency and neurotoxicity of QUAN-0808 when administered orally, compared to ip administration. No neurotoxicity was found after oral administration at the very high dose of 4500 mg/kg. Therefore, the PI for oral administration exceeded 187.5, which was both higher than the PI of ip administration and better than the PI of most of clinical drugs. The anticonvulsant activity of QUAN-0808 was also investigated against seizures induced chemically by the administration of PTZ, 3-MP, ISO and THIO to confirm the above findings and to test the possible mechanisms involved in the activity of QUAN-0808. The QUAN-0808 protected against seizures induced by PTZ, ISO, THIO and 3-MP, with  $ED_{50}$  values of 22.8, 9.5, 2.2 and 1.5 mg/kg, respectively. The QUAN-0808 had positive effects in both electrical and chemical tests. These animal models are usually considered suitable for identifying anticonvulsant activity against a variety of seizures.<sup>1,9</sup> The chemical models of seizure, such as PTZ-induced seizures, represent models of 'myoclonic seizure' that produce clonic and tonic seizures. The MES test, which is the most frequently used animal model, was used for the identification of anticonvulsant activity of drugs against 'grand mal' seizures. QUAN-0808 was able to control the seizures induced by PTZ, ISO, THIO and 3-MP, suggesting that it exhibits a varieties of anticonvulsant activity in animal models of partial and generalized epilepsy. PTZ and ISO are believed to induce seizures by inhibiting  $\gamma$ -aminobutyric acid (GABA) neurotransmission. GABA is the main inhibitory neurotransmitter in the

brain, and it is strongly implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity promotes and facilitates seizures<sup>3,4,10</sup> while enhanced GABAergic neurotransmission inhibits or attenuates seizures. The QUAN-0808 inhibits or attenuates PTZ- and ISO-induced seizures in mice by enhancing GABAergic neurotransmission. 3-MP and THIO are competitive inhibitors of the enzyme glutamate decarboxylase (GAD) that decreases the formation and level of GABA in the brain. The QUAN-0808 showed greater anticonvulsant activity, lower neurotoxicity, and a larger PI than the clinical drug carbamazepine in the MES test. QUAN-0808 elicited significant antagonism toward seizures induced by PTZ, 3-MP, THIO and ISO.

## ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITY

Inflammation is a common pathophysiology of various diseases and the most protective response of the body to noxious stimulation. Although there are different possible causes of inflammation, the basic pathological changes and clinical symptoms are quite similar. The basic pathological changes like partial response for microvascular leakage of blood components, tissue and cell degeneration, necrosis, hyperplasia and repair, whereas the clinical symptoms include redness, swelling, fever, pain and dysfunction.<sup>11-14</sup> Chronic and uncontrolled inflammation can be regular in various diseases, like cardiovascular diseases, autoimmune rheumatoid arthritis, systemic lupus erythematosus, cancer and Alzheimer's and Parkinson's diseases.<sup>13,15,16</sup>

The efficiency and low toxicity are essential for anti-inflammatory drugs. QUAN-0808 was tested for the anti-inflammatory and analgesic effects. The QUAN-0808 (100, 200, 400 mg/kg) and indomethacin (Indo) significantly decreased xylene-induced ear edema by 33.3, 37.5, 46.6, and 45.1%, respectively, decreased Carr-induced paw edema at 1, 2, 4 h after Carr injection, and decreased the prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and nitric oxide (NO) levels on the edema paw at 4 h after Carr injection; QUAN-0808 (100,200, 400 mg/kg), and aspirin (200 mg/kg) appreciably decreased acetic acid-induced capillary permeability hyperactivity model by 26.7, 28.7, 32.3 and 29.1%, respectively, and decreased the numbers of acetic acid-induced writhing response in 15 min by 40.4, 53.6, 66.4, and 64.5%, respectively. Morphine (10 mg/kg) considerably increased the

latency of the hotplate response by 136.5, 117.4, 67.5, and 22.7%, respectively, at 30, 60, 90, 120 min after intraperitoneal injection of morphine; however, QUAN-0808 (100,200 and 400 mg/kg) did not produce significantly antinociceptive effects in the hot plate test, its antinociceptive action occurs via peripheral rather than a central-acting mechanism. The QUAN-0808 produced potential anti-inflammatory and peripheral antinociceptive effects. Antinociceptive effects of QUAN-0808 were related to its anti-inflammatory activity in a dose-dependent manner. Inflammation is a peripheral process, QUAN-0808 exerted peripheral effects. The peripheral effect mechanisms of QUAN-0808 may be related to a decrease in the formation of PGE<sub>2</sub>, NO, bradykinin and other inflammatory mediators.<sup>17</sup>

The anti-inflammatory and antinociceptive activities of QUAN-0808 are using various animal models to clarify the pain and inflammation relieving effects. Xylene-induced ear edema is used to evaluate the anti-inflammatory activities of compounds. As a chemical agent, xylene can cause the release of inflammatory mediators, including histamine, kinins and fibrinolytic enzyme. The release of inflammatory mediators leads to increased local capillary permeability and inflammatory cell infiltration, resulting in acute exudative inflammatory ear edema.<sup>18-20</sup> The xylene-induced mouse ear edema assay that QUAN-0808 appreciably inhibited xylene-induced ear edema in a dose dependent manner. This effect may be produced by a reduction in the levels of histamine, kinins and other inflammatory mediators. The injection of Carr into mice produces a typical biphasic edema related with the formation of numerous inflammatory mediators, including bradykinin, prostaglandins (PGs), NO and cytokines.<sup>21</sup> Development of edema induced by Carr is correlated with the early exudates stage of inflammation, which is one of the important processes of inflammatory pathology. The injection of Carr into the paw of a rodent, several mediators are sequentially released, including histamine, serotonin and bradykinin in the initial phase (0-1 h), followed in the later phase (1-6 h) by an increase in the production of PGs through the activation of cyclooxygenase-2 (COX-2) and the release of NO.<sup>12,21-23</sup> During the inflammatory process, large amounts of the proinflammatory mediators, NO and PGE<sub>2</sub>, are produced by inducible iNOS and

COX-2. The reactive oxygen species, NO and PGE2 are considered as inflammatory factors and play major roles in the damage of tissues by inflammation.<sup>24,25</sup> The injection of Carr into the mic paw induced the release of bradykinin, which induced the biosynthesis of PGs and other autacoids that are liable for the formation of the inflammatory exudates.<sup>26,27</sup> PGs play a major role in the inflammatory response and it is recognized that an array of organizations excited by physical, chemical and biological factors lead to the formation and release of a range of PGs. As arachidonic acid (AA) metabolites from the COX pathway with extensive range of biological activities, PGE2 and PGI2 are major and closely related with the inflammatory response. Their proinflammatory function mainly involves dilation of blood vessels, sensitization of pain nerve endings and fever. NO is an important mediator and regulator of inflammatory response.<sup>23</sup> In an inflammatory response, NO is over formed and this highly reactive molecule reacts with superoxide, leading to cytotoxicity and tissue damage. NO is also liable for vasodilatation, an increase in vascular permeability and edema formation at the site of inflammation.<sup>28</sup> The mechanism of inflammation, L-arginine-NO pathway plays a main role in Carr-induced paw edema. The subsequent production of NO maintained the edema.<sup>25,29</sup> Carr-induced paw edema model results in the production of NO. The Carr testis highly sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs) and has been accepted as a valuable phlogistic tool for examining new drug therapies. The anti-inflammatory effects of QUAN-0808 on paw edema induced by Carr mice and detected the levels of NO and PGE2 in the paw edema, to understand the anti-inflammatory mechanism of QUAN-0808 and it produced a dose-dependent inhibition. QUAN-0808 and Indo (an indole arylacetic acid class of nonselective COX-inhibitor and widely prescribed NSAID<sup>18,22</sup>) greatly inhibited the development of edema and they showed anti-inflammatory effects in Carr induced mice paw edema. The levels of NO and PGE2 were reduced extensively in a dose dependent manner by treatment with 100, 200 and 400 mg/kg of QUAN-0808. The anti-inflammatory mechanism of QUAN-0808 may be related to the L-arginine-NO and PGE2 pathways. QUAN-0808 appreciably inhibited the development of the edema as induced

by xylene or Carr, the anti-inflammatory mechanism of QUAN-0808 may also be related to a reduction of histamine, kinins and other inflammatory mediators. The acetic acid-induced peritonitis test model is used for the study of acute peritoneal inflammation.<sup>19</sup> The impact of any drugs tested on capillary permeability in mice can be observed by measuring the optical density of the peritoneal exudates. QUAN-0808 considerably inhibited the leakage of dye, in spite of the increase in capillary permeability. The increase in vascular permeability induced by acetic acid is known to correspond to the initial exudative inflammation<sup>28</sup> and its inhibition may contribute to the reduction of edema formation.<sup>14</sup>

Two different analgesic testing methods were used to identify the possible peripheral and central effects of the test compound. The acetic acid-induced abdominal constriction and hot plate methods were used to evaluate peripheral and central activity, respectively. Acetic acid induces inflammatory pain by inducing capillary permeability<sup>12,19</sup>, whereas hot plate induced pain indicates narcotic involvement.<sup>20</sup> Acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to NSAIDs and opioids. Acetic acid-induced writhing is a visceral pain model which, despite its low specificity, has been widely used for the test of peripheral antinociceptive activity because of its high sensitivity.<sup>30</sup>

Oral use of QUAN-0808 considerably reduced the number of writhings induced by acetic acid in mice and the activity was comparable to 200 mg/kg Aspirin (po). The QUAN-0808 inhibited acetic acid-induced abdominal constrictions in mice, thereby exhibiting an antinociceptive effect. Acetic acid stimulates the release of several mediators, including bradykinin, substance P and PGs.<sup>26,31</sup> The antinociceptive activity confirmed by QUAN-0808, it inhibited these mediators and the activation of chemo-sensitive nociceptors that contribute to the progress of inflammatory pain. The hot-plate test is usually used to evaluate narcotic analgesia. Though the central and peripheral analgesics respond by inhibiting the number of contractions provoked by chemical pain stimuli, only the central analgesics increase the time of response in the hot plate test, because the hot plate is a specific central antinociceptive test in which opioid agents exert their analgesic effects via supraspinal and spinal



receptors.<sup>32,33</sup> In the hot plate test, treatment with morphine (10 mg/kg, ip) caused a noticeable raise in the latency time of the animals, whereas QUAN-0808 (100–400 mg/kg, po) did not notably alter latency time relative to the control. Thus, at the doses of 100–400 mg/kg, QUAN-0808 protected mice against chemically-induced noxious stimuli, but not thermal-induced noxious stimuli. The antinociceptive effects of QUAN-0808 occur via peripheral rather than centrally-acting mechanisms. The peripheral antinociceptive effect of QUAN-0808 may be mediated via the inhibition of PGs, bradykinin, substance P and other inflammatory mediators. QUAN-0808 produced potential anti-inflammatory and peripheral antinociceptive effects assessed using both chemical and thermal methods, the antinociceptive effects of QUAN-0808 were related to its anti-inflammatory activity in a dose-dependent manner. Therefore, as inflammation is a peripheral process, it is suggested that QUAN-0808 exerted peripheral effects. The peripheral effect mechanisms of QUAN-0808 may be related to a decrease in the production of PGE<sub>2</sub>, NO, bradykinin and other inflammatory mediators.

### ANTICOAGULANT AND ANTITHROMBOTIC ACTIVITY

Thrombosis is a main complication that could be fatal in acute or chronic cardio-cerebral vascular diseases. The development of new drugs for anticlotting and the prevention of thrombosis and cardiovascular diseases are clinically significant. The anticoagulant and antithrombotic effects of QUAN-0808, Bleeding time, clotting time, and serum calcium ion (Ca<sup>2+</sup>) concentration were assessed in mice, whereas arterio-venous thrombus weight and plasma prothrombin time were evaluated in rats, and platelets Ca<sup>2+</sup> influx was determined in rabbit. Daily oral administration of Q808 at 25, 50, or 100 mg/kg for 3 days significantly delayed bleeding time and clotting time in mice compared with controls. The Q808 administration at 50 mg/kg significantly reduced experimental thrombus weight by 62.6% and delayed plasma prothrombin time by 58.7% in rats, whereas 50 and 100 mg/kg of Q808 daily significantly increased serum Ca<sup>2+</sup> concentration in mice. QUAN-0808 at 0.2, 0.4, and 0.8 mg/mL considerably inhibited thrombin induced Ca<sup>2+</sup> influx in rabbit platelets. QUAN-0808 at 25-200 mg/kg daily exerts anticoagulant and antithrombotic

effects, and its mechanisms of action may involve both the intrinsic and extrinsic coagulation pathways that inhibit certain coagulation factors and platelet functions.<sup>34</sup>

### ANTIDEPRESSANT ACTIVITY

The antidepressant-like effect of QUAN-0808, in the tail suspension test (TST), the Q808 at 10 and 20 mg·kg<sup>-1</sup> dose produced a statistically significant reduction of 26% and 29% in immobility after the third administration, while imipramine 10 mg·kg<sup>-1</sup> 42%. The Q808 at 5, 10 and 20 mg·kg<sup>-1</sup> produced a reduction by 24%, 27% and 35%, respectively, after the seventh administration, whereas imipramine 28%. In forced swimming test (FST), QUAN-0808 10 and 20 mg·kg<sup>-1</sup> produced a reduction of 28% and 29%, and imipramine 27% after the third administration; and QUAN-0808 5, 10 and 20 mg·kg<sup>-1</sup> produced a reduction of 25%, 27% and 30%, whereas imipramine 27% after the seventh administration. QUAN-0808 5, 10 and 20 mg·kg<sup>-1</sup> and imipramine did not considerably change MAO activity in FST or the examining activity of the mice in the open-field test. In the reserpine test, QUAN-0808 20 mg·kg<sup>-1</sup> produced a reduction of 55% in the degree of ptosis, and imipramine 60%; QUAN-0808 10 and 20 mg·kg<sup>-1</sup> produced an antagonism in the hypothermia at 2, 3 and 4 h by 25%, 32%, 27% and 33%, 28%, 29%, while imipramine antagonized the hypothermia by 29%, 28% and 21%. The QUAN-0808 has an antidepressant-like effect on mice.<sup>35</sup>

### CONCLUSION

Above study suggested that QUAN-0808 was appreciably exhibited anticonvulsant, anti-inflammatory, analgesic, anticoagulant, antithrombotic and antidepressant activities.

### REFERENCES

1. Zhang L, Guan LP, Sun XY, Wei CX, Chai KY, Quan ZS. Synthesis and anticonvulsant activity of 6-alkoxy-[1,2,4]triazolo[3,4-a]phthalazines. *Chem Biol Drug Des*, 2009, 73, 313–319.
2. Chen J, Sun XY, Chai KY, Lee JS, Song MS, Quan ZS. Synthesis and anticonvulsant evaluation of 4-(4-alkoxyphenyl)-3-ethyl-4H-1,2,4-triazoles as open-chain analogues of 7-alkoxy-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolines. *Bioorg Med Chem*, 2007, 15, 6775–6781.
3. El-Gazzar AR, El-Enany MM, Mahmoud MN. Synthesis, analgesic, anti-inflammatory, and antimicrobial activity of some novel pyrimido[4,5-b]quinolin-4-ones. *Bioorg Med Chem*, 2008 16, 3261–3273.

4. Israili ZH. Clinical pharmacokinetics of angiotensin II (AT1) receptor blockers in hypertension. *J Hum Hypertens*, 2000, 14, Suppl 1, S73–86.
5. Sun X-Y, Wei C-X, Deng X-Q, Sun Z-G, Quan Z-S. Evaluation of the anticonvulsant activity of 6-(4-chlorophenoxy)-tetrazolo[5,-a] phthalazine in various experimental seizure models in mice. *Pharmacological Reports*, 2010, 62, 273-277.
6. Al-Soud YA, Al-Masoudi NA, FerwanahAel-R. Synthesis and properties of new substituted 1,2,4-triazoles: potential antitumor agents. *Bioorg Med Chem*, 2003, 8, 1701-1708.
7. Sun XY, Zhang L, Wei CX, Piao HR, Quan ZS. Characterization of the anticonvulsant activity of doxepin in various experimental seizure models in mice. *Pharmacol Rep*, 2009, 61, 245–251.
8. Castel-Branco MM, Alves GL, Figueiredo IV, Falcao AC, Caramona MM. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. *Methods Find ExpClinPharmacol*, 2009, 31, 101-106.
9. Talarek S, Listos J, Fidecka S. Role of nitric oxide in the development of tolerance to diazepam-induced motor impairment in mice. *Pharmacol Rep*, 2008, 60, 475-482.
10. Karnik AV, Malviya NJ, Kulkarni AM, Jadhav BL. Synthesis and in vitro antibacterial activity of novel heterocyclic derivatives of 18-nor-equilenin. *Eur J Med Chem*, 2006, 41, 891–895.
11. Li CM, Wang MH. Anti-inflammatory effect of the water fraction from hawthorn fruit on LPS-stimulated RAW 264.7 cells. *Nutr Res Pract*, 2011, 2, 101–106.
12. Santos EN, Lima JCS, Noldin VF, Cechinel-Filho V, Rao VSN, Lima EF, Schmeda-Hirschmann G, Paulo T. Sousa Jr.<sup>IV</sup>; Domingos T.O. Martins. Anti-inflammatory, antinociceptive, and antipyretic effects of methanol extract of *Cariniana-rubrastem* bark in animal models. *An Acad Bras Ciênc*, 2011, 2, 557–566.
13. Sarkar D, Fisher PB. Molecular mechanisms of aging associated inflammation. *Cancer Lett*, 2006, 236, 13-23.
14. Silva LMCM, Lima V, Holanda ML, Pinheiro PG, Rodrigues JAG, Lima MEP, Benevides NMB. Antinociceptive and anti-inflammatory activities of lectin from marine red alga *Pterocladiaella-capillacea*. *Biol Pharm Bull*, 2010, 5, 830–835.
15. Huang GJ, Huang SS, Lin SS, Shao YY, Chen CC, Hou WC, Kuo YH. Analgesic effects and the mechanisms of anti-inflammation of ergostatrien-3b-ol from *Antrodiaacam-phoratasubmerged* whole broth in mice. *J Agric Food Chem*, 2010, 58, 7445–7452.
16. Karin M, Lawrence T, Nizet V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell*, 2006, 124, 823–835.
17. Yu H-L, Zhang F, Lan, T, Quan Z-S. Effects of 6-(4-chlorophenoxy)-tetrazolo[5,1-a]phthalazine on Anticoagulation in Mice and the Inhibition of Experimental Thrombosis in Rats. *J Cardiovasc Pharmacol*, 2014, 64(6), 560–566.
18. Abdullah GZ, Abdulkarim MF, Salman IM, Ameer OZ, Yam MF, Muttee AF, Chitneni M, Elrashid S Mahdi, Mahiran Basri, Munavvar A Sattar, and Azmin M Noor In vitro permeation and in vivo anti-inflammatory and analgesic properties of nanoscaled emulsions containing ibuprofen for topical delivery. *Int J Nanomed*, 2011, 6, 387–396.
19. Barros WM, Rao VSN, Silva RM, Lima JCS, Martins DTO. Anti-inflammatory effect of the ethanolic extract from *Bowdichiavirgili-oidesH.B.K* stem bark. *An Acad Bras Ciênc*, 2010, 3, 609–616.
20. Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev*, 2001, 53, 597–652.
21. Okusada K, Nakamoto K, Nishida M, Fujita-Hamabe W, Kamiya K, Mizushima Y, Satake T, Tokuyama S. The antinociceptive and anti-inflammatory action of the CHCl<sub>3</sub>-soluble phase and its main active component, damnacanthal, isolated from the root of *Morindacitrifolia*. *Biol Pharm Bull*, 2011, 1, 103–107.
22. Jedinak A, Dudhgaonkar S, Wu QL, Simon J, Sliva D. Anti-inflammatory activity of edible oyster mushroom is mediated through the inhibition of NF-κB and AP-1 signaling. *Nutr J*, 2011, 10, 52.
23. Salvemini D, Ischiropoulos H, Cuzzocrea S. Roles of nitric oxide and superoxide in inflammation. *Methods MolBiol*, 2003, 225, 291–303.
24. Botting RM. Vane's discovery of the mechanism of action of aspirin changed our understanding of its clinical pharmacology. *Pharmacol Rep*, 2010, 62, 518–525.
25. Yun KJ, Kim JY, Kim JB. Inhibition of LPS-induced NO and PGE<sub>2</sub> production by asiatic acid via NF-κB inactivation in RAW264.7 macrophages: possible involvement of the IKK and MAPK pathways. *IntImmunopharmacol*, 2008, 3, 431–441.
26. Capone ML, Tacconelli S, Rodriguez LG, Patrignani P. NSAIDs and cardiovascular disease: transducing human pharmacology results into clinical read-outs in the general population. *Pharmacol Rep*, 2010, 62, 518-525.
27. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP. Prostaglandins as modulators of immunity. *Trends Immunol*, 2002, 3, 144–150.
28. MullaWA, Kuchekar SB, Thorat VS, Chopade AR, Kuchekar BS. Antioxidant, antinociceptive and anti-inflammatory activities of ethanolic-aextract of leaves of *Alo-casiaindica* (Schott.). *J Young Pharm*, 2010, 2, 137-143.
29. Yoon WJ, Ham YM, Kim KN, Park SY, Lee NH, Hyun CG, Lee WJ. Anti-inflammatory activity of brown alga *Dictyotadichotomain* murine macrophage RAW264.7 cells. *J Med Plant Res*, 2009, 3, 1–8.
30. Crain SM, Shen KF. Acute thermal hyperalgesia elicited by low-dose morphine in normal mice is blocked by ultra-low dose naltrexone, unmasking potent opioid analgesia. *Brain Res*, 2001, 88, 75–82.
31. Chu C, Huang Y, Chen YF, Wu JH, Rahman K, Zheng HC, Qin LP. Anti-nociceptive activity of aqueous fraction from the MeOH extracts of *Paederiascandensin* mice. *J Ethnopharmacol*, 2008, 118, 177–180.
32. da Silveira e Sá Rde C, de Oliveira LE, Nóbrega FF, Bhattacharyya J, de Almeida RN. Antinociceptive and toxicological effects of *Dioclea grandiflora* seed pod in mice. *J Biomed Biotechnol*, 2010, 10, 1–6.
33. Mbiantcha M, Kamanyi A, Teponno RB, Tapondjou AL, Watcho P, Nguielefack TB: Analgesic and anti-inflammatory properties of extracts from the bulbils of *Dioscoreabul-biferaL. varsativa* (Dioscoreaceae) in mice and rats. *Evid Based Complement Alternat Med*, 2011, 10, 1–9.
34. Yu H-L, Feng-Zhang, Li Y-J, Gong G-H, Quan Z-S. Anti-inflammatory and antinociceptive effects of 6-(4-chlorophenoxy)-tetrazolo[5,1-a] phthalazine in mice. *Pharmacological Reports*, 2012, 64 (5), 1155-1165.
35. Feng Z, Jiang H-C, Chen X-R, Ma Z, Yu H-L, Quan Z-S. Antidepressant-like effect of 6-(4-chlorophenoxy)-tetrazolo[5,1-a] phthalazine in mice. *Chinese J Pharmacol & Toxicol*, 2013, 27(5), 10.3867/j.issn.1000-3002.2013.05.003.