Anti-inflammatory Activity of 3-Thiazolyl Coumarins

[1]Uzma Salar, [1]Khalid Mohammed Khan*, [2]Almas Jabeen, [1]Bakhtawar, [2]Aisha Faheem, [3]Muhammad Taha, [1]Farman Ali, [1]Shazia Syed, [1]Kanwal, [1]Syed Moazzam Haider, and [4]Shahnaz Perveen

[1]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

[2]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

[3]Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, P.O. Box 31441, Dammam, Saudi Arabia.

[4]PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Khalid.khan@iccs.edu*

(Received on 1st January 2016, accepted in revised form 23rd August 2017)

Summary: 3-Thiazolyl coumarins **1-33** along with coumarin scaffold (IC_{50} = 5.2 ± 0.2 μg/mL) were evaluated for *in vitro* antiinflammatory activity. Activity of compounds was investigated by looking their influence on oxidative burst activity of zymosan stimulated whole blood phagocytes by using a luminol enhanced chemiluminescence technique. Ibuprofen was used as standard drug (IC_{50} = 54.2 ± 9.2 μM). Four 3-thiazolyl coumarin derivatives **9** (IC_{50} = 31.0 ± 2.5 μg/mL), **13** (IC_{50} = 27.1 ± 4.2 μg/mL), **18** (IC_{50} = 5.6 ± 2.6 μg/mL), and **29** (IC_{50} = 1.9 ± 1.0 μg/mL) out of thirty-three demonstrated antiinflammatory activity as compared to the standard ibuprofen (IC_{50} = 11.2 ± 1.9 μg/mL). Especially, compound **29** showed many folds better activity as compared to coumarin and standard ibuprofen. Structure-activity relationship was also established. It is worth-mentioning that active analogs **9**, **13**, **18**, and **29** were found to be non-toxic on NIH-3T3 mouse fibroblast cell line.

Keywords: Coumarin, Thiazole, Antiinflammatory activity, Synthesis, Ibuprofen, Non-toxic.

Introduction

Coumarin is an important class of heterocyclic compounds and reported to exhibit diversified pharmacological activities with less toxicity. A continuous and increased attention of medicinal chemists is observed due to their beneficial effects on human health [1, 2]. Being a privileged scaffold, coumarins are reported to have bioactivities such as anticoagulant [3], anti-HIV [4, 5], antioxidant [6, 7], antibacterial [8], antiinflammatory [9, 10], anticancer [11], and dyslipidemic [12] activities. Among these properties, cytotoxic effects were most extensively examined [13, 14]. Coumarin derivatives were also reported as lipid-lowering agents [15], triplet sensitizers [16], vasorelaxant [17], free radical scavengers, and lipid peroxidation inhibitors [18].

Thiazole is associated with noteworthy medicinal importance due to its potential as antiviral [19], pesticidal [20], antiinflammatory [21], antitumor [22], antituberculosis [23], and enzyme inhibitory activities [24]. They have also been reported to have broad application in the cure of allergies [25], hypertension [26], and schizophrenia [27]. Likewise, thiazole substituted coumarin nucleus showed potential pharmacological activities [28] such as anticancer, antimicrobial [29], anticonvulsant [30], anti-inflammatory, and analgesic activities [31].

Phagocytic cells usually involve in the first line defense by the immune system in response to acute microbial diseases and leads to inflammation [32]. Hereditary defects of these phagocytic cells produce infections such as chemotaxis on sites of inflammation in different pathologic processes [33]. In inflammatory process, neutrophils go through a process of respiratory burst which results in increased consumption of oxygen then undergoes reduction *via* NADPH oxidase to superoxide anion and harmful reactive oxygen species (ROS). Superoxide dismutase (SOD) catalyzes the reaction of O_2 molecules to form harmful hydrogen peroxide, dioxygen and hydroxyl radical ($OH^{\bullet}$) [34]. ROS are the basis of different pathologies and needs to inhibit this chronic inflammation in patients of pathetic immune system [35-37]. Non-steroidal antiinflammatory drugs (NSAIDs) such as aspirin, ibuprofen, flufenamic acid, indomethacin, and phenylbutazone, are extensively used to treat most of the inflammatory disorders [38-40], however, long term usage leads to various side effects such as nephrotoxicity, gastric ulceration, and bleeding. According to literature, NSAIDs may play an important part to prevent reactive oxygen species mediated damage [41]. Most of the NSAIDs *e.g.* aspirin, indomethacin, phenylbutazone, ibuprofen, and flufenamic acid are acidic in nature have pKa

*To whom all correspondence should be addressed.

values in the range of 3.5-5.5 [42]. The acidic nature which is the main cause of adverse side effects associated with these marketed drugs have prompted us to work on non-acidic pharmacophore in search of better antiinflammatory agent with less or no side effects.

Many heterocyclic compounds have previously been reported to demonstrate antiinflammatory activity [42-47], as well as natural and synthetic coumarins were also found to scavenge reactive oxygen species [36, 37]. On the basis of all evidences, we decided to screen series of coumarins **1-33** incorporated with thiazole ring to check their anti-inflammatory activity. Thus, this study reports the *in vitro* anti-inflammatory activities of 3-thiazolyl coumarins **1-33**. All the synthetic compounds gave satisfactory results in physico-chemical analyses.

Experimental

Oxidative Burst Assay

Luminol-enhanced chemiluminescence assay was carried out as reported by Helfand *et. al.* [48]. Concisely, diluted whole blood in HBSS++ (Hanks Balanced Salt Solution, containing $MgCl_2$ and $CaCl_2$) from healthy human volunteer (25 μL) was incubated with three different concentrations (1, 10 and 100 μg/mL) of compounds (25 μL), each in triplicate. Control wells contained HBSS++ and cells, but no compounds. Test was carried out in white half area of 96 well plates which was also incubated in the thermostat chamber of luminometer at 37 $^{\circ}$C for 15 minutes. After incubation, serum opsonized zymosan (25 μL) and intracellular reactive oxygen species detecting probe (25 μL), luminol were added into each well, except blank wells containing only HBSS++. The level of ROS was recorded in luminometer, in terms of relative light units (RLU). Ibuprofen was used as standard.

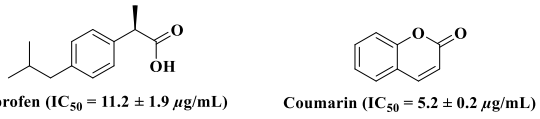
MTT Cytotoxicity assay

Cytotoxicity of 3-thiazolyl coumarins on NIH-3T3 fibroblast cells (ATCC, Manassas, USA) was screened by using the standard MTT colorimetric assay [49]. Briefly, 100 μL of 5×10^4 cells/mL in DMEM supplemented with 10% FBS were plated into 96-wells flat bottom plate and incubated overnight at 37 $^{\circ}$C in 5% CO_2. Three different concentrations of test compound (1, 10 and 100 μg/mL) were added to the plate in triplicates and incubated for 48 h. 50 μL of 0.5 mg/mL MTT was added to each well and plate was then further incubated for 4 hours. MTT was aspirated and 100 μL of DMSO was then added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 540 nm, using spectrophotometer (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxic activity was recorded as concentration causing 50% growth inhibition (IC_{50}) for 3T3 cells.

Results and Discussion

In Vitro Antiinflammatory Activity

3-Thiazolyl coumarins **1-33** along with simple coumarin scaffold (IC_{50} = 5.2 ± 0.2 μg/mL) were evaluated for *in vitro* antiinflammatory activity in whole blood oxidative burst assay. Four 3-thiazoly coumarin derivatives **9** (IC_{50} = 31.0 ± 2.5 μg/mL), **13** (IC_{50} = 27.1 ± 4.2 μg/mL), **18** (IC_{50} = 5.6 ± 2.6 μg/mL), and **29** (IC_{50} = 1.9 ± 1.0 μg/mL), out of thirty-three, displayed good activity when compared to the standard ibuprofen (IC_{50} = 11.2 ± 1.9 μg/mL) (Table-1). All active compounds **9**, **13**, **18**, and **29** were found to be non-toxic when tested on NIH-3T3 mouse fibroblast cell line.



Ibuprofen (IC_{50} = 11.2 ± 1.9 μg/mL) Coumarin (IC_{50} = 5.2 ± 0.2 μg/mL)

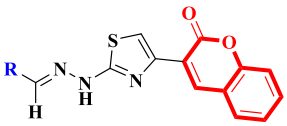
Fig. 1: *In vitro* antiinflammatory activity of ibuprofen and coumarin

Structure-Activity Relationship (SAR)

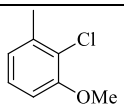
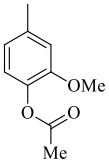
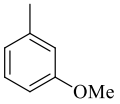
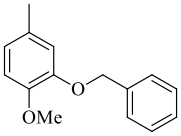
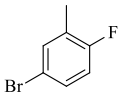
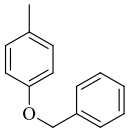
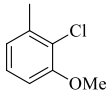
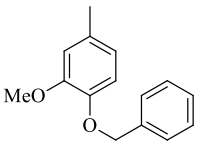
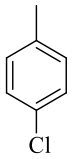
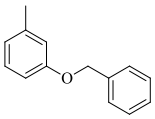
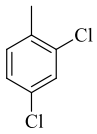
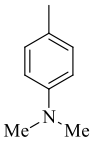
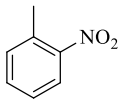
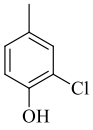
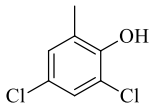
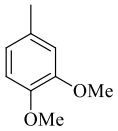
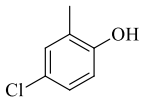
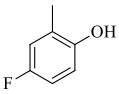
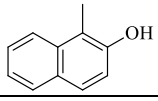
Limited SAR proposed that different substitutions at ring R responsible for the varying activities. Compound **29** (IC_{50} = 1.9 ± 1.0 μg/mL) having indole ring as R, was found to be the most potent compound of this library which indicated that indole ring is important for the excellent activity. It is worth-noting that compound **29** was found to be many folds active than coumarin scaffold (IC_{50} = 5.2 ± 0.2 μg/mL). Compound **18** (IC_{50} = 5.6 ± 2.6 μg/mL) was found to be the second most potent compound and have 3-OMe and 4-OH groups at ring R, demonstrated two-fold better activity than the standard as well as comparable activity to coumarin ring (IC_{50} = 5.2 ± 0.2 μg/mL) (Figure-2).

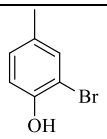
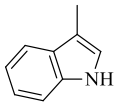
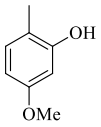
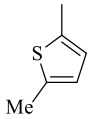
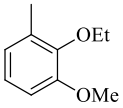
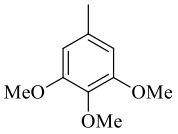
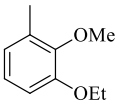
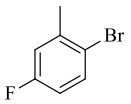
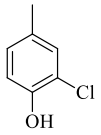
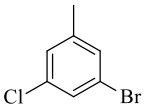
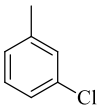
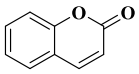
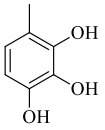
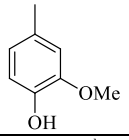
.

Table-1: *In Vitro* Antiinflammatory Activity of Thiazolyl Coumarin Derivatives and Coumarin Scaffold **1-33.**



(1-33)
3-Thiazolyl coumarins

Compounds	R	IC_{50} ± SD[a]	Compounds	R	IC_{50} ± SD[a]
1		**NA[b]**	**19**		**NA[b]**
2		**NA[b]**	**20**		**NA[b]**
3		**NA[b]**	**21**		**NA[b]**
4		**NA[b]**	**22**		**NA[b]**
5		**NA[b]**	**23**		**NA[b]**
6		**NA[b]**	**24**		**NA[b]**
7		**NA[b]**	**25**		**NA[b]**
8		**NA[b]**	**26**		**NA[b]**
9		**31.0 ± 2.5**	**27**		**NA[b]**
10		**NA[b]**	**28**		**NA[b]**

11		**NA[b]**	**29**		**1.9 ± 1.0**
12		**NA[b]**	**30**		**NA[b]**
13		**27.1 ± 4.2**	**31**		**NA[b]**
14		**NA[b]**	**32**		**NA[b]**
15		**NA[b]**	**33**		**NA[b]**
16		**NA[b]**	**Coumarin**		**5.2 ± 0.2**
17		**NA[b]**			
			Standard[c] = Ibuprofen		**11.2 ± 1.9**
18		**5.6 ± 2.6**			

IC_{50}[a] (Mean ± Standard Deviation); NA[b] (Not Active); Standard[c] (Inhibitor for antiinflammatory activity).

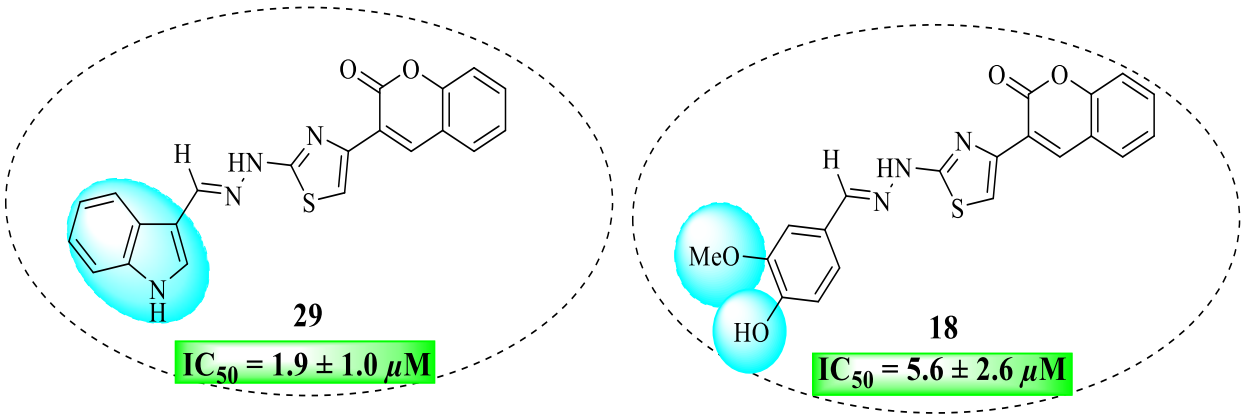


Fig. 2: Structure-activity relationship of compounds **18** and **29**.

Comparison of activity of compound **18** (IC_{50} = 5.6 ± 2.6 μg/mL) with structurally similar analogs such as **2** and **5** which have the acetylated and benzylated hydroxy group at *para* position of ring R, loss of activity observed as shown in Figure-3. Similarly, compounds **11** and **15**, those have bromo and chloro, respectively, instead of methoxy at *meta* position, also showed no antiinflammatory activity. It demonstrated that methoxy group at *meta* and hydroxy at *para* position played their role in the activity (Figure-3).

Compounds **9** (IC_{50} = 31.0 ± 2.5 μg/mL) having substitution of OMe group at positions 3 and 4 of ring R, displayed good activity. On comparison of its activity with structurally similar analogs such as **3** and **5** which have benzyloxy group instead of one of the methoxy group showed no activity. Similarly compound **20** which only lacks one methoxy group also found to be not active (Figure-4).

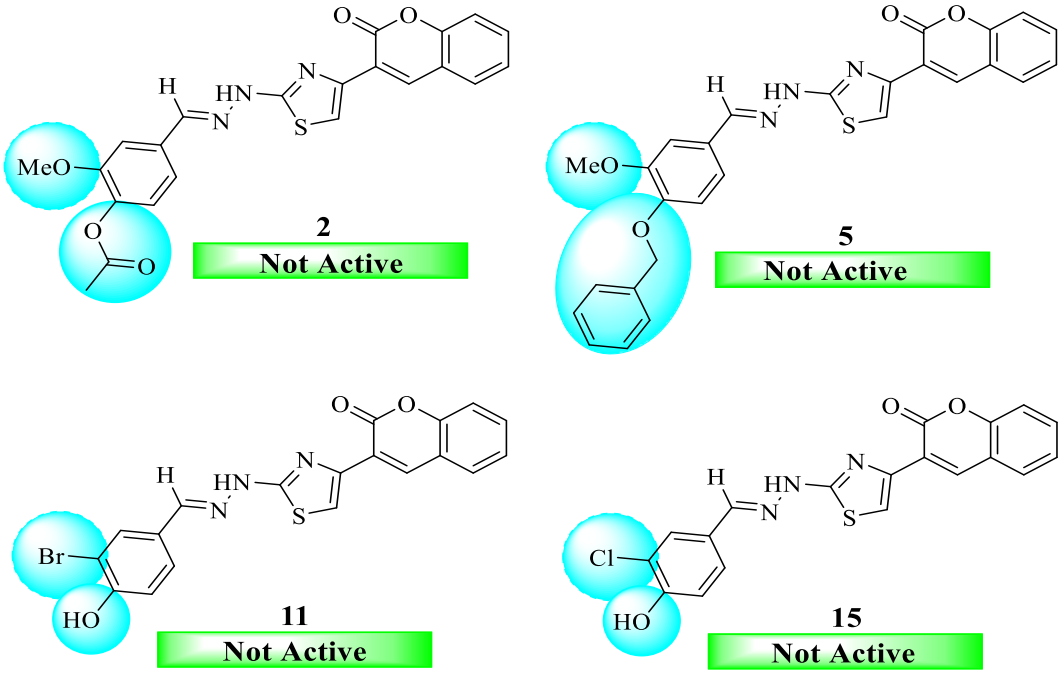


Fig. 3: Structure-activity relationship of compounds **2**, **5**, **11**, and **15**.

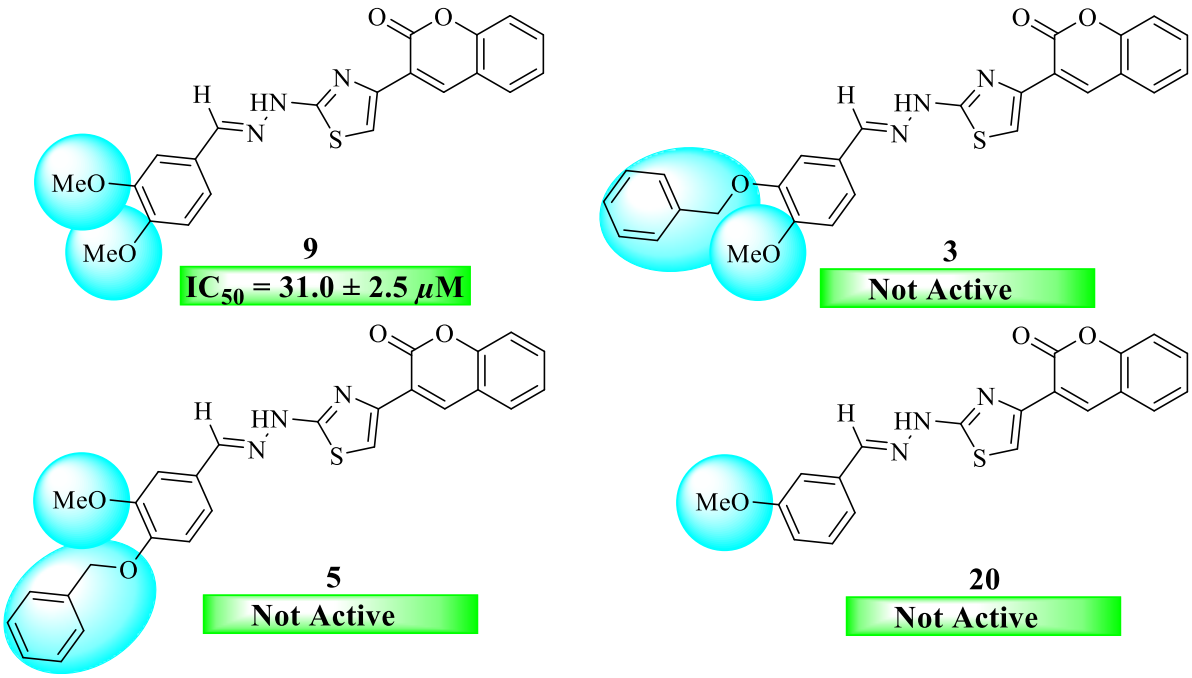


Fig. 4: Structure-activity relationship of compounds **3**, **5**, **9**, and **20**.

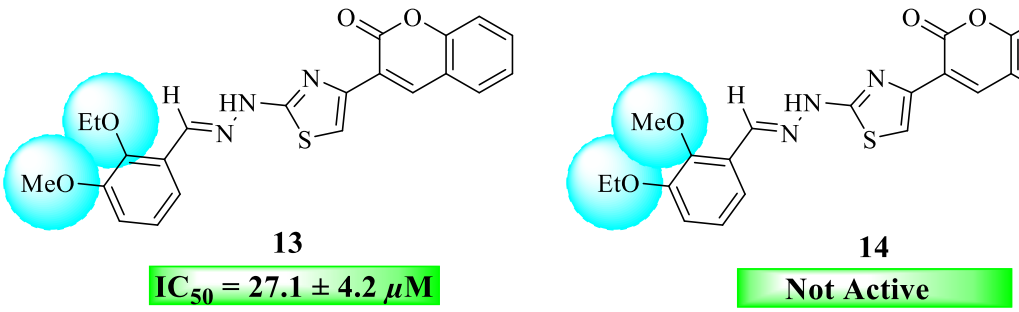


Fig. 5: Structure-activity relationship of compounds **13** and **14**

Compound **13** (IC_{50} = 27.1 ± 4.2 μg/mL) also showed good activity and have OEt and OMe substitution at positions 2 and 3, respectively. Switching the position of methoxy with ethoxy substituents brought the complete loss of activity (Figure-5

On the basis of limited SAR, it was found that all three compounds **9**, **13**, and **18** possess OMe at position 3 of ring R. It showed that OMe substitution at 3 position of ring R played an important part in the antiinflammatory activity.

Conclusion

Conclusively, 3-thizolylcoumarins **1-33** were synthesized and evaluated for *in vitro* antiinflammatory activity. Analogs **9**, **13**, **18**, and **29** were showed good antiinflammatory potential when compared with the standard ibuprofen (IC_{50} = 11.2 ± 1.9 μg/mL) and also found to be non-toxic on NIH-3T3 mouse fibroblast cell line. Analogs **18** (IC_{50} = 5.6 ± 2.6 μg/mL) and **29** (IC_{50} = 1.9 ± 1.0 μg/mL) were found to be the most potent analogs and can serve as lead compounds for future research.

Acknowledgements

The authors are thankful to Higher Education Commission (HEC) Pakistan for financing the Project No. 20-1910 under National Research Program for Universities.

References

1. J. R. S. Hoult and M. Paya, Pharmacological and Biochemical Actions of Simple Coumarins: Natural Products with Therapeutic Potential. *Gen. Pharma.: Vascul. Sys*. **27**, 713 (1996).
2. V. S. Koneni, K. Abdhesh, K. Manoj, S. Jayanta and S. Sudhir, Synthesis and *In Vitro* Evaluation of Novel Coumarin-Chalcone Hybrids as Potential Anticancer Agents. *Bioorg. Med. Chem. Lett.*, **20**, 7205 (2010).
3. A. G. Kidane, H. Salacinski, A. Tiwari, K. R. Bruckdorfer and A. M. Seifalian, Anticoagulant and Antiplatelet Agents: their Clinical and Device Application (s) Together with Usages to Engineer Surfaces. *Biomacromol*. **5**, 798 (2004).
4. T. Ma, L. Liu, H. Xue, L. Li, C. Han, L. Wang, Z. Chen and G. Liu, Chemical Library and Structure-Activity Relationships of 11-demethyl-12-oxo Calanolide A Analogues as Anti-HIV-1 Agents. *J. Med. Chem*. **51**, 1432 (2008).
5. C. Spino, M. Dodier and S. Sotheeswaran, Anti-HIV Coumarins from *Calophyllum* Seed Oil. *Bioorg. Med. Chem. Lett*. **8**, 3475 (1998).
6. C. A. Kontogiorgis and L. D. Hadjipavlou-Litina, Synthesis and Biological Evaluation of Novel Coumarin Derivatives with a 7-Azomethine Linkage. *Bioorg. Med. Chem. Lett*. **14**, 611 (2004).
7. C. Kontogiorgis and D. Hadjipavlou-Litina, Biological Evaluation of Several Coumarin Derivatives Designed as Possible Anti-Inflammatory/Antioxidant Agents. *J. Enz. Inhib. Med. Chem*. **18**, 63 (2003).
8. G. Appendino, E. Mercalli, N. Fuzzati, L. Arnoldi, M. Stavri, S. Gibbons, M. Ballero and A. Maxia, Antimycobacterial Coumarins from the Sardinian Giant Fennel (*Ferula communis*). *J. Nat. Prod*. **67**, 2108 (2004).
9. N. S. Reddy, M. R. Mallireddigari, S. Cosenza, K. Gumireddy, S. C. Bell, E. P. Reddy and M. V. R. Reddy, Synthesis of New Coumarin 3-(*N*-aryl) Sulfonamides and their Anticancer Activity. *Bioorg. Med. Chem. Lett*. **14**, 4093 (2004).
10. L. Z. Chen, W. W. Sun, L. Bo, J. Q. Wang, C. Xiu, W. J. Tang, J. B. Shi, H. P. Zhou and X. H. Liu, New Arylpyrazoline-Coumarins: Synthesis and Anti-Inflammatory Activity. *Eur. J. Med. Chem*. **138**, 170 (2017).
11. I. Kempen, D. Papapostolou, N. Thierry, L. Pochet, S. Counerotte, B. Masereel, J. M. Foidart

and M. J. Reboud-Ravaux, A. Noel, B. Pirotte, 3-Bromophenyl 6-acetoxymethyl-2-oxo-2*H*-1-benzopyran-3-carboxylate inhibits cancer cell invasion *in vitro* and tumour growth *in vivo*. *British J. Cancer*, **88**, 1111 (2003).

12. K. V. Sashidhara, J. N. Rosaiah, A. Kumar, G. Bhatia and A. K. Khanna, Synthesis of Novel Benzocoumarin Derivatives as Lipid Lowering Agents. *Bioorg. Med. Chem. Lett*. **20**, 3065 (2010).
13. I. Kostova, Synthetic and Natural Coumarins as Cytotoxic Agents. *Curr. Med. Chem-Anti-Cancer Agents*, **5**, 29 (2005).
14. M. A. Musa and J. S. Cooperwood, A Review of Coumarin Derivatives in Pharmacotherapy of Breast Cancer. *Curr. Med. Chem*. **15**, 2664 (2008).
15. G. R. Madhavan, V. Balraju, B. Mallesham, R. Chakrabarti and V. B. Lohray, Novel Coumarin Derivatives of Heterocyclic Compounds as Lipid-Lowering Agents. *Bioorg. Med. Chem. Lett*. **13**, 2547 (2003).
16. J. L. R. Williams, D. P. Specht and S. Farid, Ketocoumarins as Photosensitizers and Photoinitiators. *Poly. Eng. Sci*. **23**, 1022 (1983).
17. J. R. S. Hoult and M. Paya, Pharmacological and Biochemical Actions of Simple Coumarins: Natural Products with Therapeutic Potential. *Gen. Pharma.: Vascul. Sys.* **27**, 713 (1996).
18. A. Mora, M. Paya, J. L. Rios and M. Alcaraz, Structure-Activity Relationships of Polymethoxyflavones and other Flavonoids as Inhibitors of Non-Enzymic Lipid Peroxidation. *Biochem. Pharmacol*. **40**, 793 (1990).
19. F. C. Spector, L. Liang, H. Giordano, M. Sivaraja and M. G. Peterson, Inhibition of Herpes Simplex Virus Replication by a 2-amino thiazole via Interactions with the Helicase Component of the UL5-UL8-UL52 Complex. J. Virology, **72**, 6979 (1998).
20. Z. Fan, Z. Shi, X. Liu, Z. Fan and Y. Ai, Faming Zhuanli Shenqing Gongkai Shuomingshu, 17 (2006) Chinese patent:. CNXXEV CN 1810808 A 20060802.
21. B. Holla, K. Malini, B. Rao, B. Sarojini and N. Kumari, Synthesis of Some New 2, 4-disubstituted thiazoles as Possible Antibacterial and Anti-Inflammatory Agents. *Eur. J. Med. Chem*. **38**, 313 (2003).
22. H. I. El-Subbagh, H. A. Ashraf and L. Jochen, Synthesis and Antitumor Activity of Ethyl 2-substituted-aminothiazole-4-carboxylate analogs. *Archiv der Pharmazie*, **332**, 137 (1999).
23. K. M. Sytnik, I. Ye, S. Bylov and N. Kovalenko, Zhurnal Organichnoi ta Farmatsevtichnoi Khimii, **1**, 71 (2003).
24. J. Das, J. Furch, C. Liu, R. Moquin, J. Lin, S. Spergel, K. McIntyre, D. Shuster, K. O'Day and B. Penhallow, Discovery and SAR of 2-amino-5-(thioaryl) thiazoles as Potent and Selective Itk Inhibitors. *Bioorg. Med. Chem. Lett*. **16**, 3706 (2006).
25. S. Shigenaga, T. Manabi, H. Matsuda, T. Fujii and J. Hiroi, Synthesis and Pharmacological Properties of *N*-[4-[4-(1*H*-indol-3-yl)-piperidinoalkyl]-2-thiazolyl] alkanesulfonamides as Novel Antiallergic Agents. *Chem. Pharma. Bull*. **41**, 1589 (1993).
26. W. Patt, H. Hamilton, M. Taylor, M. Ryan, D. Taylor Jr., C. Connolly, A. Doherty, S. Klutchko and I. Sircar, Structure-Activity Relationships of a series of 2-amino-4-thiazole-containing renin inhibitors. *J. Med. Chem*. **35**, 2562 (1992).
27. M. Ohkabu, A. Kuno, H. Sakai and H. Takasugi, Studies on Cerebral Protective Agents. VIII. Synthesis of 2-aminothiazoles and 2-thiazolecarboxamides with Anti-Anoxic Activity. *Chem. Pharma. Bull*. **43**, 1497 (1995).
28. P. V. Kumar, K. M. Reddy and V. R. Rao, Synthesis of Some 7-methyl-3-(2-oxo-2*H*-chromen-3-yl)-5*H*[1, 3] thiazolo [3, 2-a] pyrimidin-5-ones. *Ind. J. Chem. Sec*. B, **47**, 759 (2008).
29. A. Kamal, S. Adil, J. Tamboli, B. Siddardha and U. Murthy, Synthesis of Coumarin Linked Naphthalimide Conjugates as Potential Anticancer and Antimicrobial Agents. *Lett. Drug Des. Discov*. **6**, 201 (2009).
30. N. Siddiqui, M. Arshad and S. A. Khan, Synthesis of Some New Coumarin Incorporated Thiazolyl Semicarbazones as Anticonvulsants. *Acta. Pol. Pharm. Drug Res*. **66**, 161 (2009).
31. R. Kalkhambkar, G. Kulkarni, H. Shivkumar and R. Rao, Synthesis of Novel Triheterocyclic Thiazoles as Anti-Inflammatory and Analgesic Agents. *Eur. J. Med. Chem.* **42**, 1272 (2007).
32. C. A. Kontogiorgis and D. J. Hadjipavlou-Litina, Synthesis and Antiinflammatory Activity of Coumarin Derivatives. *J. Med. Chem*. **48**, 6400 (2005).
33. L. Chang-Hui, H. Yen-Ju and L. Yin-Chou, Celecoxib Simulates Respiratory Burst Through Pertussis Toxin-Sensitive G-Protein, a Possible Signal for β 2-Integrin Expression on Human Neutrophils. *Eur. J. Pharma.* **484**, 29 (2004).
34. P. K. Narayanan, W. O. Carter, P. E. Ganey, R. A. Roth, S. L. Voytik-Harbin and J. P. Robinson, Impairment of Human Neutrophil Oxidative Burst by Polychlorinated Biphenyls: Inhibition of Superoxide Dismutase Activity. *J. Leukcyte Bio*. **63**, 216 (1998).

35. K. M. Khan, M. Khan, M. Ali, M. I. Qadir, S. Parveen, A. Karim and M. I. Choudhary, Superoxide Respiratory Burst Inhibitory Activity of *Bis*-Schiff Bases of Isatins. *J. Chem. Soc. Pak.* **35**, 987 (2013).
36. M. Paya, B. Halliwell and J. R. S. Hoult, Interactions of a Series of Coumarins with Reactive Oxygen Species: Scavenging of Superoxide, Hypochlorous Acid and Hydroxyl Radicals. *Biochem. Pharmacol.* **44**, 205 (1992).
37. U. Salar, K. M. Khan, A. Jabeen, A. Faheem, M. I. Fakhri, S. M. Saad, S. Perveen and M. Taha, Coumarin Sulfonates: As Potential Leads for ROS Inhibition. *Bioorg. Chem.* **69**, 37 (2016).
38. F. A. Omar, N. M. Mahfouz and M. A. Rahman, Design, Synthesis and Antiinflammatory Activity of Some 1, 3, 4-oxadiazole Derivatives. *Eur. J. Med. Chem*. **3**, 819 (1996).
39. R. T. Schoen and R. J. Vender, Mechanisms of Nonsteroidal Anti-Inflammatory Drug-Induced Gastric Damage. *Am. J. Med.* **86**, 449 (1989).
40. J. G. Lombardino and E. H. Wiseman, Preparation and Antiinflammatory Activity of Some Nonacidic Trisubstituted Imidazoles. *J. Med. Chem*. **1**, 1182 (1974).
41. P. L. Hiller, K. O. Hodd and R. L. Wilson, Antiinflammatory Drugs: Protection of a Bacterial Virus as an *In Vitro* Biological Measure of Free Radical Activity. *Chem. Biolog. Inter*. **47**, 293 (1983).
42. K. M. Khan, Zia-Ullah, M. A. Lodhi, S. Jalil, M. I. Choudhary and Atta-ur-Rahman, Synthesis and Anti-Inflammatory Activity of Some Selected Aminothiophene Analogs. *J. Enz. Inhib. Med. Chem*. **21**, 139 (2006).
43. E. Marchetti, G. Mattalia and V. Rosnati, A New Class of Analgetic-Antiinflammatory Agents. 2-Substituted 4, 5-Diphenyloxazoles. *J. Med. Chem*. **11**, 1092 (1968).
44. H. Kano, I. Adachi, R. Kito and K. Hirose, Isoxazoles. XVIII. Synthesis and Pharmacological Properties of 5-aminoalkyl-and 3-aminoalkylisoxazoles and Related Derivatives. *J. Med. Chem*. **10**, 411 (1967).
45. J. Szmuszkovicz, E. M. Glenn, R. Y. Heinzelman, B. Hester, Jr.and G. A. Youngdale, Synthesis and Antiinflammatory Activity of 2, 3-*bis*(*p*-methoxyphenyl) Indole and Related Compounds. *J. Med. Chem.* **9**, 527 (1966).
46. L. Almirante, L. Polo, A. Mugnaini, E. Provinciali, P. Iiugarli, A. Biancotti, A. Gamba and W. Murmann, Derivatives of imidazole. I. Synthesis and Reactions of Imidazo [1, 2-α] Pyridines with Analgesic, Antiinflammatory, Antipyretic, and Anticonvulsant Activity. *J. Med. Chem.* **8**, 305 (1965).
47. J. G. Lombardino and E. H. Wiseman, Preparation and Antiinflammatory Activity of Some Nonacidic Trisubstituted Imidazoles. *J. Med. Chem.* **1**, 1182 (1974).
48. S. L. Helfand, J. E. R. O. M. E. Werkmeister and J. C. Roder, Chemiluminescence Response of Human Natural Killer Cells. I. The Relationship Between Target Cell Binding, Chemiluminescence, and Cytolysis. *J. Exp. Med*. **156**, 492 (1982).
49. A. Jabeen, M. A. Mesaik, S. U. Simjee, Lubna, S. Bano and S. Faizi, Anti-TNF-α and Antiarthritic Effect of Patuletin: A Rare Flavonoid from *Tagetes patula*. *Int. immunopharm.* **36**, 232 (2016).