"Synthesis and Biological Evaluation of Novel Pyrazole derivatives as Potential Anticancer Agents"

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ABSTRACT: Colorectal cancer (CRC) and melanoma are major causes of cancer-related death worldwide. Signal transducer and activator of transcription 3 (STAT3) plays a pivotal role in cancer development. Inhibition STAT3 signaling is a promising strategy for treating CRC and melanoma. *Huai-Hua-San* (HHS), a traditional Chinese medicine (TCM) formula comprising Sophorae Flos (SF) and Gardeniae Fructus (GF), Several compounds, such as quercetin and genipin, occurring in SF and/or GF have been reported to have anti-CRC and anti-melanoma properties and inhibit STAT3 signaling. In this study, we evaluated the anti-CRC and anti-melanoma effects of an ethanolic extract of HHS (HHSE), and investigated the involvement of STAT3 signaling in these effects pharmacology studies showed that 17 bioactive compounds are potential anti-CRC components of HHS. To determine which of the 17 compounds can inhibit Src/STAT3 signaling, we performed molecular docking to identify Src binding compounds. Results showed that ISO exhibited high affinity of binding to Src kinase domain. ISO has been reported to possess anti-CRC properties and to induce autophagy in Caco2 CRC cells. Immunoblotting showed that ISO inhibited Src/STAT3 signaling in CRC cells and tumors, and over-activation of STAT3 diminished the cytotoxic effects of ISO in HCT116 cells. Moreover, ISO induced autophagy via inhibiting AKT/mTOR signaling in CRC cells. Blocking autophagy using an autophagy inhibitor chloroquine (CQ) or 3-methyladenine (3-MA) enhanced the cytotoxic effect of ISO in CRC cells. Compared to ISO alone, ISO plus CQ or 3-MA exerted more potent apoptotic effects in CRC cells. In a HCT116 cell-bearing mouse model, 3-MA enhanced the effects of ISO in suppressing tumor growth.

Keywords: Pyrazole derivatives , Anticaner agents, Gardeniae Fructus; Colorectal; STAT3 signaling;

INTRODUCTION

The synthesis of pyrazole and isoxazole derivatives has been the subject of consistent interest because of the widespread applications of such heterocycles in pharmaceutical as well as agrochemical industry. Pyrazoles and isoxazoles are well known for their anti-inflammatory activity. In order to develop potent anticaner agents, a novel series of 3-(1H-indol-3-yl)-2,3,3a,4-tetrahydrothiochromeno[4,3-c]pyrazole derivatives were synthesized. Structures of all compounds were confirmed. MTT assay has been employed to study antiproliferative activity of these compounds with four human cancer cell lines [5] and a normal cell line L929. Most of these compounds showed potential anticancer activity and low cytotoxicity on normal cell in vitro. Numerous compounds containing pyrazole and isoxazole moieties have been shown to exhibit anticancer, integrin avb3 receptor antagonists, antimicrobial, molluscicidal, glycine agonists, 20-hydroxyeicosatetraeonic acid (20-HETE) synthase inhibitory, histone deacetylase 3 and 8 (HDAC3 and HDAC8) gene inhibitory, and antioxidant activities [6]. Furthermore, 1,5-benzoxazepine derivatives have been recognized as novel microtubule-targeting agents. Some substituted benzoxazepine and benzothiazepine were found to exhibit antipsychotic and anticonvulsant activity. Moreover, there are some reports for benzothiazepine and benzodiazepine derivatives as anti-inflammatory agents. Several benzothiazepine derivatives have been reported to have potential calcium channel blocker activity. Benzodiazepines were found to show sentrin- specific protease 1 (SENP1) and p53-mouse double minute 2 (p53-MDM2) inhibitory activities. On the other hand, the heterocyclic compounds containing aryl sulfonate moiety are known to exhibit marked antimicrobial activity [7]. In addition, they are possessed of potential papillomavirus microbicidal, anti-human immunodeficiency virus-1, antineoplastic and anticancer activity[8]. These observations have encouraged us to synthesize some new pyrazole, isoxazole, benzoxazepine, benzothiazepine and benzodiazepine derivatives containing aryl sulfonate moiety via the cyclo-condensation reaction in aqueous medium under microwave irradiation conditions by an efficient and general one pot-three component procedure in the hope to evaluate their potential antimicrobial and anti-inflammatory activities.[9]

Cancer is a public health problem, and the second leading cause of death worldwide. It is a chronic health condition whose incidence and mortality rate are markedly roaring worldwide. In 2020, there were approximately 19.3 million new cancer cases and 10 million deaths occurred globally. In Hong

Kong, colorectal cancer (CRC) is the commonest cancer, and melanoma is one of the most aggressive cancers.

CRC epidemiology

More than 1.8 million new CRC cases and 862,000 deaths were estimated to occur in 2021, accounting for 10% of all cancer cases and deaths worldwide [10]. The incidence of CRC ranks the third while its mortality ranks the second. CRC incidence rates in developed countries are about 4-fold higher than that in developing countries. However, there is less variation in mortality rates due to higher fatality in developing countries. The incidence of CRC varies largely depending on geographic regions, with the highest rates being reported in Europe, Australia/New Zealand, and Northern America; and the lowest rates being reported in Africa, South Asia and Central Asia [11]. The incidence rates of CRC are steadily rising in many countries and territories, such as Eastern Europe, Central Asia, South East Asia and South America. As one of the largest developing countries, China has been experiencing a stunning increase in the incidence of CRC, from 12.10/100,000 in 1990 to 22.42/100,000 in 2017[12]. CRC is a major public health issue and incurs a heavy economic burden on the society and individuals [13]. In Hong Kong, CRC is the commonest cancer. It accounts for 17.0% of all new cancer cases in 2018. Primary prevention remains an important approach to reduce the increasing global burden of CRC [14]. The risk factors of CRC are modifiable and nonmodifiable. The modifiable risk factors include obesity, diet, smoking, alcohol consumption, physical activity and so on. The nonmodifiable risks factors include sex, age, familial adenomatous polyposis, lynch syndrome, inherited genetic risk, type 2 diabetes, inflammatory bowel disease and so on [15]. In the past 20 years, improvements in diagnostic technologies, lifestyle and therapeutic strategies contributed to an increase from 42% to 62%. Cancer incidence and mortality are rapidly growing worldwide and the world health organization alerts that, nearly 1 in 6 deaths is because of cancer disease [16]. Consequently, an important goal of today's research is the development of new, more specific chemotherapeutics and the identification of novel biological targets, especially for the most aggressive tumors [17]. Targeted cancer therapy, in which the drugs are used to specifically block the growth of cancer by interfering with molecular targets and consequently causing less damage to normal cells, has become one of the high potential cancer treatments. Several kinds of molecular targets have been focused on in recent years, including human topoisomerase[18], because of their highly over-expression in cancer cells DNA topoisomerases are unique enzymes that alter the

topology of DNA by topoisomerases. Type I DNA topoisomerases cleave one single-stranded DNA during each catalytic cycle. Type II topoisomerases break one double-stranded DNA strand, allowing another segment of duplex DNA to pass through the transient breakage before resealing the broken strand to resolve DNA knots and tangles[19]. They can relive the DNA torsional strain and stops the cell division process [20]. They transiently break one or two strands of DNA, which allow to solve various DNA topological problems generated during vital cellular processes. Because of the crucial role of topoisomerases for the maintenance and replication of DNA during proliferation, topoisomerase inhibitors are among the most potent anticancer agents to date [21]. Topoisomerase inhibitors are classified into two groups with diverse mechanisms of action: first named poisons which act by stabilizing a covalent cleavage complex between DNA and the topoisomerase enzyme, accumulation of these complexes finally induces apoptosis, while the second diverse group of catalytic inhibitors, block the activity of the enzyme to perform catalysis, impede the binding between Topo II and DNA, block hydrolysis ATP, inhibit the ATP hydrolysis, block DNA breakage. it is known that pyrazoline derivative involves in cancer therapy, due to its huge biological activities and capability of forming hydrogen bonds [22]. Many bioactive compounds contain pyrazoline as one of the key structural unit and used as anticancer [23], antifungal [24], antibacterial [25], antioxidant [26-28], anti-tubercular [29, 30], anti-inflammatory [31-33], antimalarial [34] agents. Pyrazoline also is one of the major potential entities in the field of drug discovery using heterocyclic compounds. Combining pyrazoline moiety with other pharmacophores results enhancement of biological properties and hence, synthesizing such compounds attracts the researchers to discover novel drugs [35]. Fusing pyrazoline with thiochroman moiety could enhance the bioactive nature of the resulting compound and thereby leading to explore more novel anticancer agents. Previously, we reported bisindolylalkanes which are 3,3'-diindolylmethane analogues prossessing thiochroman for the topoisomerase inhibitory activity and cytotoxicity against several human cancer cell lines [36]. In this work, we report the design, synthesis and biological studies of a novel class of 3-(1H-indol-3-yl)-2-phenyl-2,3,3a,4-tetrahydrothiochromeno[4,3-c]pyrazole compounds which fuse pyrazoline with thiochroman containing indole skeleton. The evaluation of biological activities of these compounds was carried out for topoisomerase I and II inhibitory activity, and cytotoxicity against several human cancer cell lines. Cancer refers to uncontrolled cell division/growth of cells which have the potential to spread /invade to adjoining cells or various organs of the body by means of blood stream or lymphatic system. Since few decades, cancer has been considered as the most dreadful disorder and

is the major cause of death round the globe with an ever increasing rate. It is not restricted to a specific culture or population of a particular age. Two-third of the cancer cases mainly occurs in middle and low income countries with 16.4 million cases in 2012, which is expected to rise to 21.4 million by 2030. Approximately, 14.1 million new cancer cases come into light every year causing 15.7% deaths which nearly account to 8.8 million people. Males were found to be affected with commonly prostate, lung, stomach and colorectal cancer whereas females were found to be affected with breast, lung, cervical and colorectal cancer. Acute lymphoblastic leukemia and brain tumors are found affecting children round the globe whereas in the African continent, children were affected with non-Hodgkin lymphoma. The threat of cancer upsurges considerably with age and several cancers occur more frequently in developed countries .

Classification of Cancer

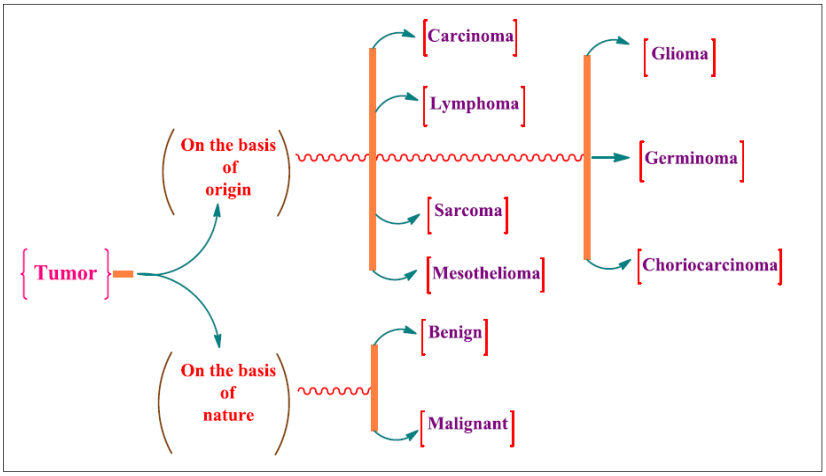
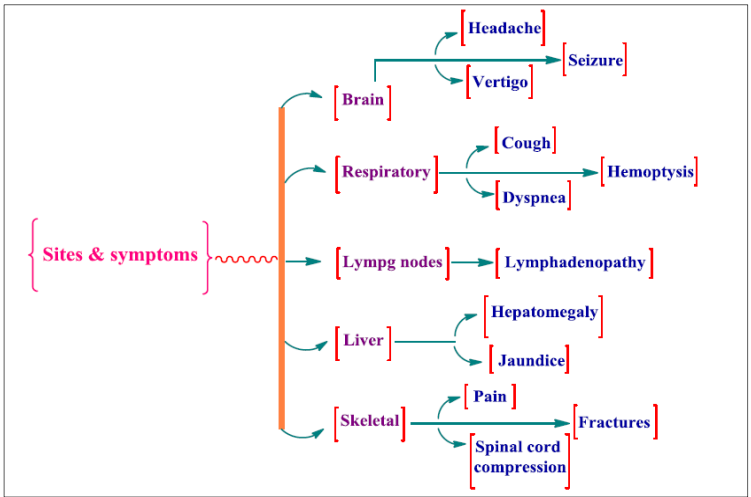


Figure no,1: Classification of cancer

Signs and symptoms of cancer

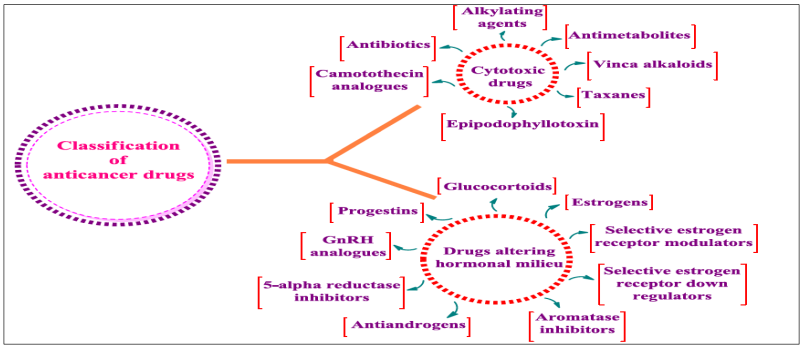
Cancer as a very silently and its symptoms are disease, progresses at most of the times



misinterpreted/confused with symptoms of any other disease. Symptoms appears when tissue/body mass grows/ulcerated in an abnormal manner. Common signs and symptoms of cancer along with the site affected in body is mentioned in Figure-2

Classification of anti-cancer drugs

Anti-cancer drugs/agents treat subjects either by killing cancerous cells or altering their growth pattern. But due to lack of specificity in majority of drugs; they are considered as one of the most toxic drugs utilized in a therapy. They are classified as cytotoxic agents and drug (Figure) altering hormonal milieu as mentioned below.

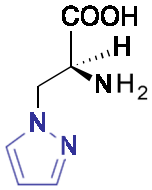


MATERIAL METHOD

4-[4-FORMYL-3-(2-NAPHTHYL)PYRAZOL-1-YL] BENZOIC ACID DERIVATIVES

The pyrazole scaffold is as an important building block in organic synthesis for designing new drugs. Pyrazole and its derivatives are an important class of well-known nitrogen heterocycles. Among

azole compounds, which contain imidazoles, isoxazoles, oxazoles, and thiazoles, pyrazoles have been identified to play an important role in medicinal chemistry.[1]



L-□-amino-□-(pyrazolyl-N)-propanoic acid

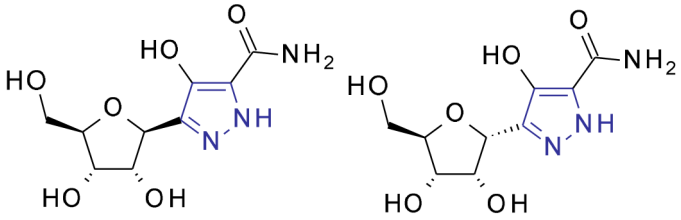


Figure 1.1. Pyrazole containing natural products (pyrazole shown in blue). Pyrazole-containing natural products including *L*-□-amino-*β*-(pyrazolyl-N)-propanoic acid (anti-diabetic),[2] pyrazofurin and pyrazofurin B (anti-viral and anti-tumor) exhibit a wide range of biological activities (**Figure 1.1**).[3] Additionally, the pyrazole ring presents as the core structure of well-established leading drugs, such as celecoxib a potent

anti-inflammatory,[4] the anti-depressant agent fezolamine,[5] the anti-obesity rimonabant,[6] and the antipsychotic CDPPB,[7] which have been used for therapeutic purposes (**Figure 1.2**).

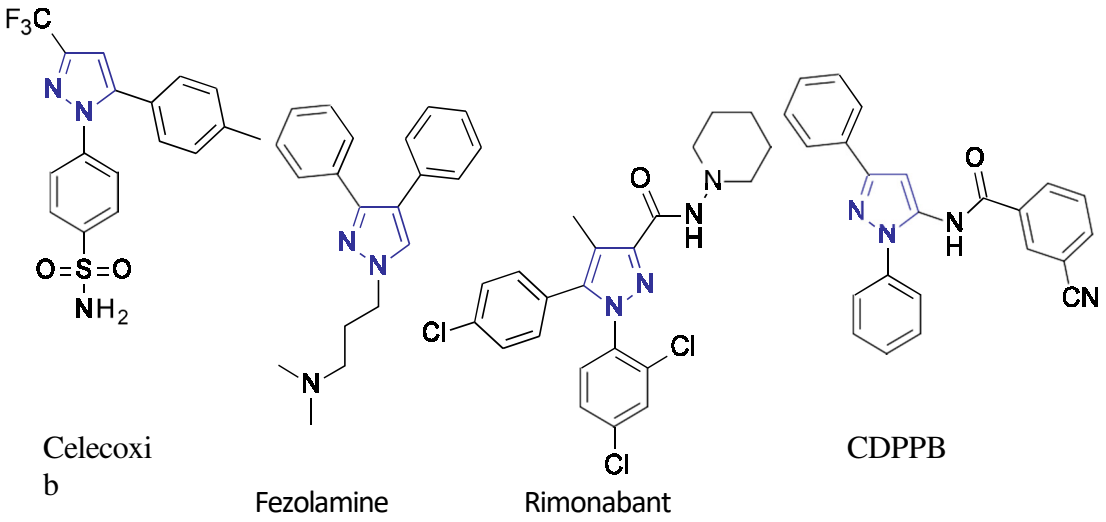


Figure 1.2. Well-established leading drugs based on pyrazole (pyrazole shown in blue).

In addition to pyrazole, hydrazone moiety is present with other functional groups in some of biologically active molecules. Other functional groups that can be combined with pyrazole are present in biologically active molecules. For example, molecules that consist of hydrazones with other functional groups show unique biological and pharmacological properties.[8] Hydrazones and its derivatives have a wide variety of biological and pharmacological properties, such as anti-bacterial,[9] anti-depressant,[10] anti- inflammatory,[11] and anti-cancer.[12] Hydrazone derivatives are present in several bioactive molecules, such as furazolidone,[13] nitrofurazone,[14] nitrofurantoin (**Figure 1.3**).[15]

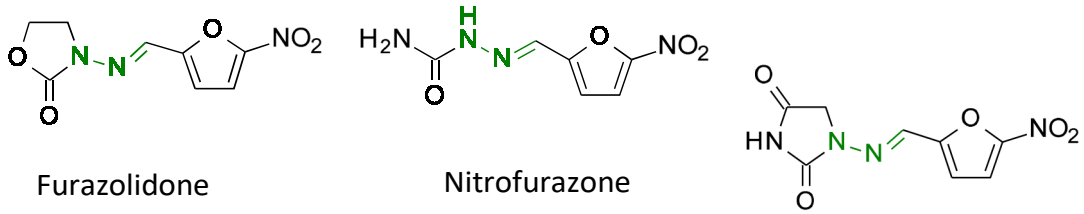
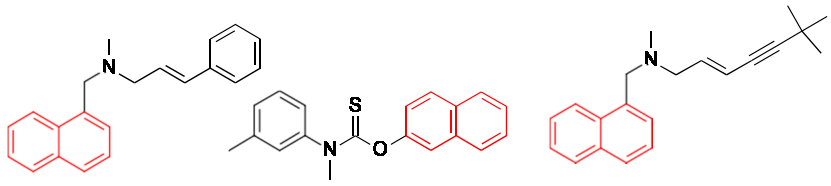


Figure 1.3. Hydrazone-containing approved drugs (hydrazone shown in green).

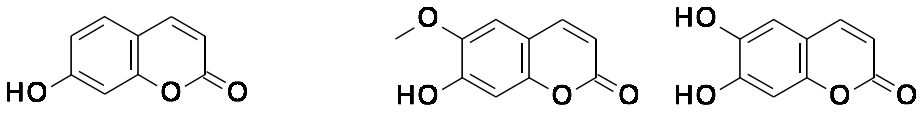
Naphthalene derivatives also display a wide range of biological activities including anti-microbial,[16] anti-inflammatory,[17] anti-diabetic,[18] antidepressant,[19] anti-cancer,[20] and anti-viral properties.[21] Several naphthalene containing drugs have been approved by FDA and are being marketed as therapeutics such as nafcillin,[22] naftifine,[23] tolnaftate,[24] and terbinofine,[25] which are prescribed for the control of microbial infections (**Figure 1.4**). There are many naphthalene-derived bioactive phytoconstituents present in nature such as rifampicin (anti-tubercular agent)[26] and patentiflorin A (anti-HIV agent) (**Figure 1.5**).[27]



SYNTHESIS OF 4-[4-FORMYL-3-(2-OXOCHROMEN-3-YL)PYRAZOL-1- YL]BENZOIC ACID DERIVATIVES

The majority of heterocycle compounds, where the most frequent substituents are nitrogen, oxygen, and sulfur, strongly impact biological activities. Therefore, heterocycle molecules constitute a core unit in current drug design as they are present in most marketed drugs. Around 80% of pharmaceutical medicines are constituted nitrogen, oxygen, or sulfur-containing heterocyclic compounds.[1] Heterocycles in natural products, which are derived from animals, plants, and microbes, have several beneficial effects on human health and are used to treat several diseases, such as cancer, brain diseases, and diabetes.[2] Coumarins, a class of organic compounds, are classified as members of the benzopyrone family, which consists of 1,2-benzopurone ring system as a basic parent scaffold. Coumarin, a heterocyclic molecule, consists of fused benzene and pyrone ring systems. Coumarin and many of its derivatives have been known in chemistry for more than a century due to their

availability, ease of extraction from plants and microorganisms, and easy synthesis in laboratories.[3]



Umbe liferone Scopoletin Aesculetin

Figure 2.1. Some of the important naturally occurring coumarins

The natural or synthetic coumarin and its derivatives have attracted considerable interest because of their various pharmacological activities.[4] Naturally-occurring coumarins are found in a wide variety of natural sources, and new coumarins are being discovered or synthesized as derivatives.[6] Various natural compounds containing the coumarin moiety are traditionally used in popular medicines all over the world. Some of the important naturally occurring coumarins are umbelliferone, scopoletin, and aesculetin, which are considered as the most common coumarins widespread in nature (**Figure 2.1**).[4] Coumarin has been the core moiety of different natural products possessing a wide spectrum of pharmacological activities including antibacterial,[7] antifungal,[8] antiviral,[8] antioxidant,[9] scavenging of reactive oxygen species (ROS),[10] anti-inflammatory,[11] and anticancer.[12]

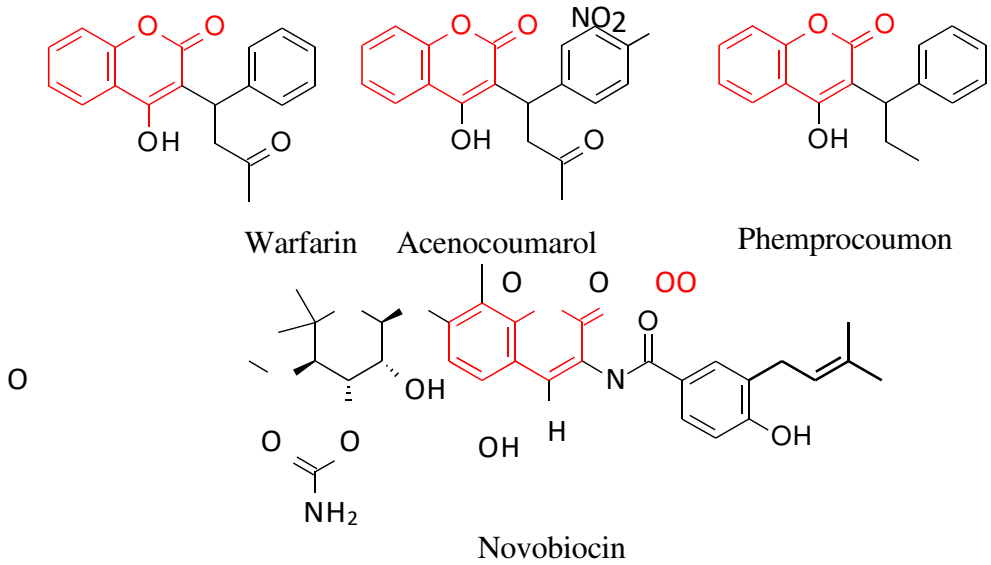
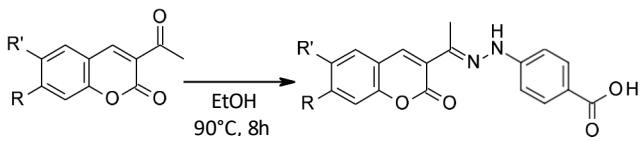
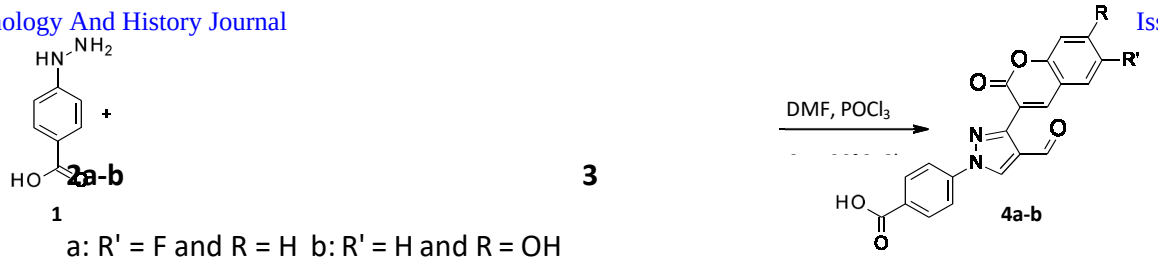


Figure 2.2. Examples of medicinally important coumarins (coumarin shown in red)

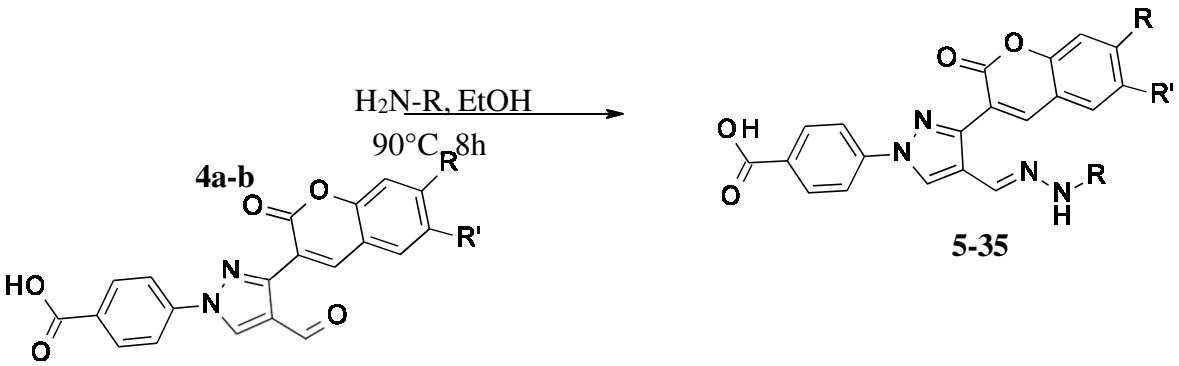
Many coumarins and their derivatives have become drugs, such as warfarin, acenocoumarol, phenprocoumon (anticoagulants),[13] and novobiocin (antibiotic, anti- cancer and/or anti-tumor) (**Figure 2.2**).[14] As a source of potential drugs, coumarin and its derivatives are plentiful candidates for drug development. When coumarin is combined with other molecules, their pharmacological and therapeutic properties change significantly.[15] Based on our lead molecules, we report the synthesis of 31 new coumarin- derived pyrazole derivatives. The new coumarin-pyrazole-hydrazone derivatives are readily synthesized by using commercially available starting materials and reagents using benign reaction conditions. All synthesized molecules were tested against several bacteria strains. Based on our reported molecules, we designed and synthesized the aldehyde derivatives (**4**) to synthesize hydrazone derivatives for antimicrobial studies.[16] A mixture of 4-hydrazinobenzoic acid (**1**, 10 mmol, 1.521 g) and 3-acetylcoumarin derivatives (**2a-b**, 10.5 mmol) in ethanol was refluxed for 8 h to obtain the hydrazone derivative (**3**) (**Scheme 2.1**). The solvent was evaporated under the reduced pressure at 60 °C, and the hydrazone derivative was further dried in vacuo. The dried product was used for further reactions without isolation or purification. The hydrazone derivative (**3**) was dissolved in *N,N*- dimethyl formamide (DMF, 30 mL) and the flask was sealed by a rubber septum. The mixture was stirred for 15 min to dissolve the solid material completely. The clear solution was stirred at 0 °C in an ice bath, and phosphorous oxychloride ($POCl_3$, 5.43 mL) was added dropwise to form Vilsmeier reagent. After 30 min, the reaction mixture was heated for eight hours at 90 °C. After the completion of the reaction, the mixture was poured onto the ice in a beaker and then stirred for 12 hours to precipitate the product, which was filtered and washed with water repeatedly until the filtrate was clear. The final product was dried under a vacuum.





Scheme 2.1. Synthesis of coumarin-substituted pyrazole-derived aldehyde.

Novel coumarin-derived hydrazones were synthesized by the reaction of the aldehyde product (**3a** and **3b**, 1 mmol) with commercially available substituted hydrazines (1.1 mmol) in ethanol (**Scheme 2.2**). Sodium acetate (1.1 mmol, 0.088 g) and acetic acid were added in case of the hydrochloride salt of the hydrazine derivatives. The resulting product was filtered and washed with ethanol (~ 15 mL) followed by washing with water (~20 mL) to get pure product.

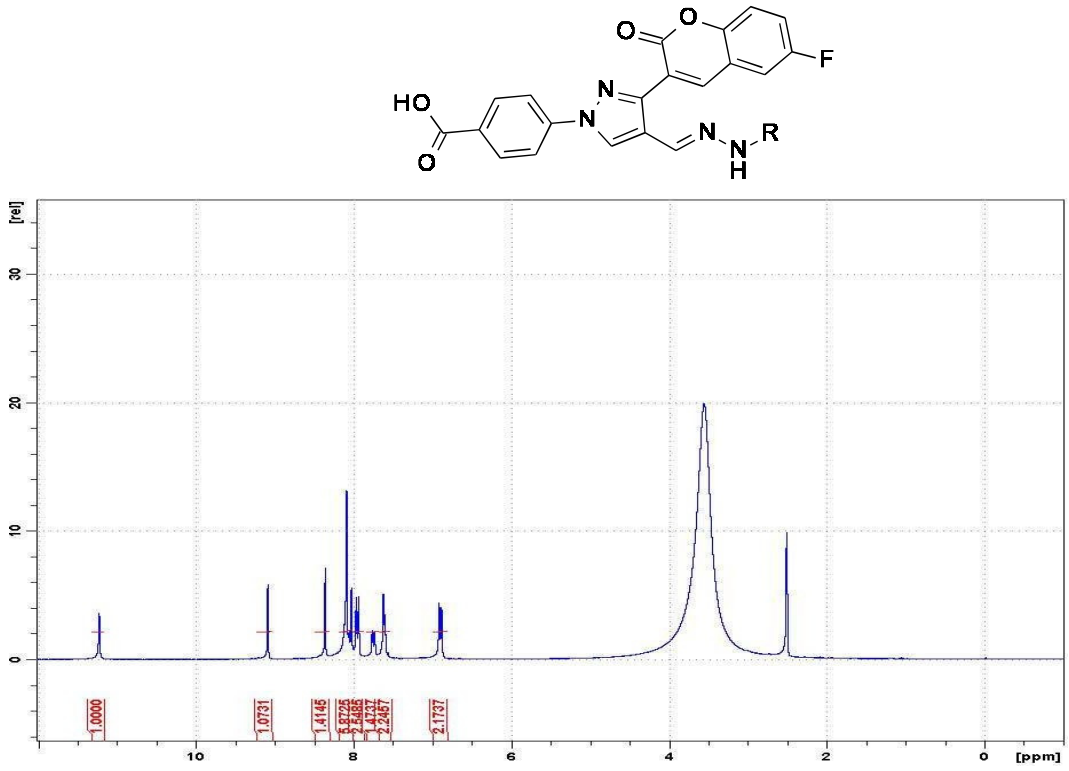


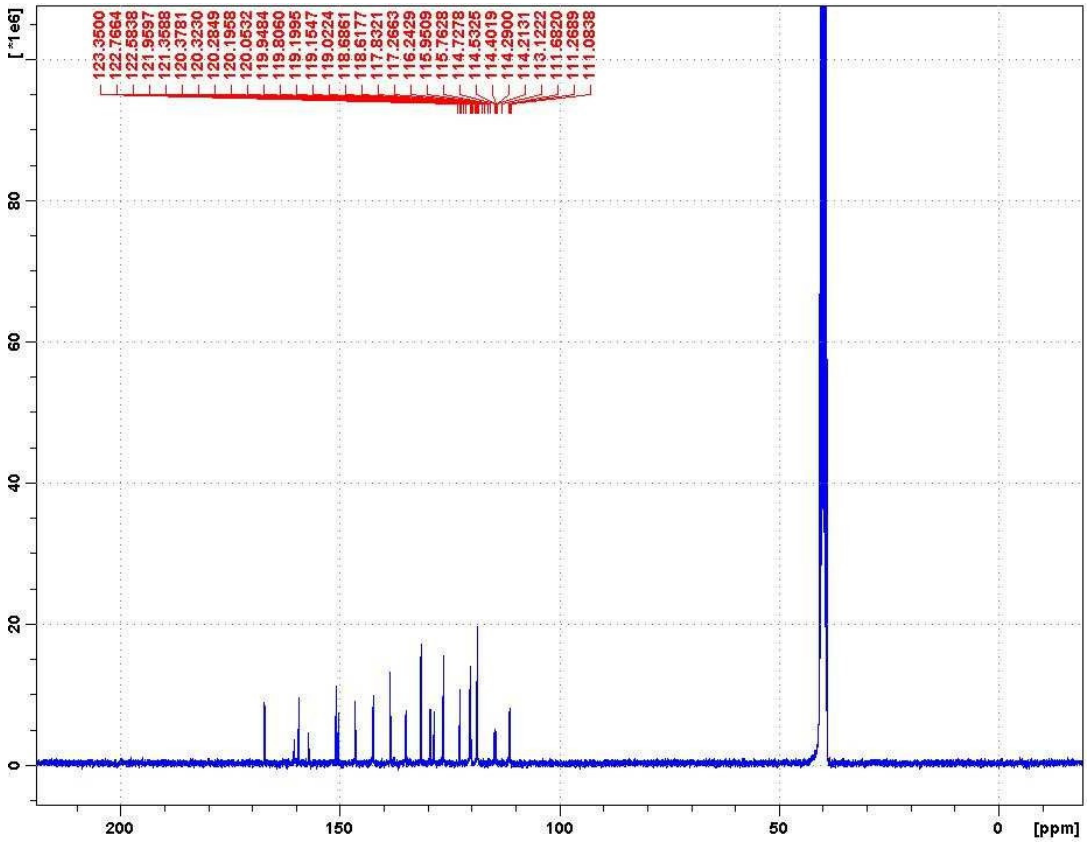
Scheme 2.2. Synthesis of coumarin-substituted pyrazole-derived hydrazone.

We have synthesized 31 new coumarin-derived hydrazones (**Table 2.1** and **2.2**). Reaction of phenylhydrazine reacted with aldehyde derivatives (**4a**-**b**) formed the products (**5** and **23**) in good yield. *N,N*-Disubstitutent also reacted with the aldehydes derivatives

(**4a**-**b**) to give the desierd compounds (**6**, **7**, and **8**) in 77%, 84%, and 82%, respectively.

Products containing electron-donating groups (**9** and **10**) were obtained in 89% and 79% yields, respectively. Withdrawing groups such as, 3-flouro substituted coumarin-derived hydarzone products (**11** and **24**) were formed in very good yeild. 3-Bromo- and 4-bromo-substituted products (**12** and **26**) were obtained in 78% and 80% yield, respectively. Also, 3-chloro- and 4-chloro-substituted products (**13** and **25**) formed in 85% and 71% yield, repectevily. Bis-halogen-substituted were formed in a good to an excellent yield. Strong withdrawing groups on the phenyl ring such as, 4-triflouromethyl-, 4-cyano-, and 4-nitro-gave the corresponding products (**19**, **20**, and **21**) in 73%, 88%, and 89% yield, respectively. Also, the products (**33** and **34**) were formed in 83% and 81% yield, repectively. *N,N*-Dimethyl coumarin-derived hydrazone (**22**) were obtained in 79% yield. Tri-flourophenyl substitutent also reacted to give 77% yield. Methyl hyrazinocarboxylate product (**35**) was obtaind in a good yield.





CONCLUSIONS

Due to the ease of synthesis without column purification or work-up, we have reported the synthesis of new hydrazone derivatives of coumarin-derived pyrazoles. We synthesized 31 new pyrazole derivatives. These new molecules were tested against several bacterial strains, and we found several molecules showed promising results.

GENERAL CONDITIONS

All of the reactions were carried out under air atmosphere in round-bottom flasks. Reagents, substrate, and solvents for reactions, recrystallizations, and deuterated solvents for ^{1}H and ^{13}C NMR spectroscopy were purchased from Fisher Scientific (Hanover Park, IL, USA.) and Oakwood chemical (Estill, SC, USA).

5 MHz, DMSO-d6): δ 167.0, 158.6 (d, ^{1}J = 239.3 Hz), 159.1, 150.3, 147.0, 146.0, 142.4, 142.1, 134.3, 131.4, 130.7 (d, ^{3}J = 22.5 Hz), 129.0, 127.9, 123.1, 120.9, 120.3, 120.1, 119.8, 118.8 (d, ^{3}J = 8.7 Hz), 118.4, 118.2, 114.4 (d, ^{2}J = 24.3 Hz), 111.1, 110.8. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{26}H_{16}ClFN_4O_4$ $[M+H]^+$ = 503.0917, 505.0890, found 503.0902, 505.0905.

4-[4-[(E)-[(4-cyanophenyl)hydrazono]methyl]-3-(7-fluoro-2-oxo-3,8a- dihydrochromen-3-yl)pyrazol-1-yl]benzoic acid (20). Yellow; (436 mg, 88%) ^{1}H NMR (300MHz, DMSO-d6): δ 10.86 (s, 1H), 9.08 (s, 1H), 8.36 (s, 1H), 8.13-8.06 (m, 4H), 7.95 (s, 1H), 7.76-7.73 (m, 1H), 7.65-7.55 (m, 2H), 7.46-7.43 (m, 2H), 6.90-6.87 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d6): δ 167.1, 159.2, 158.6 (d, ^{1}J = 239.7 Hz), 150.2, 148.8, 146.2, 142.3, 133.8, 133.0, 131.4, 129.3, 128.1, 122.7, 120.54, 120.57, 120.2 (d, ^{3}J = 9.8 Hz), 120.0, 118.7, 118.59, 118.55, 114.5 (d, ^{2}J = 24.0 Hz), 112.1, 99.3. HRMS (ESI-FTMS Mass (m/z): calcd for $C_{27}H_{16}FN_5O_4$ $[M+H]^+$ = 494.1259, found 494.1252.

REFERENCE

1. Chauhan, R., Siddiqi, A.A., Dwivedi, J., 2012. An approach to regioselective synthesis of pyrazole and isoxazole derivatives and study of their antimicrobial effect. Pharm. Chem. J. 46, 316–320.
2. Christensen, N.D., Reed, C.A., Culp, T.D., Hermonat, P.L., Howett, M.K., Anderson, R.A., Zaneveld, L.J., 2001. Papillomavirus microbial activities of high-molecular-weight cellulose sulfate, dextran sulfate and polystyrene sulfonate. Antimicrob. Agents Chemother. 45, 3427–3432.
3. A. E. Issa, N. S. Habiba, A. E. A. Wahab, Design, synthesis and biological evaluation of novel 1,2,4-triazolo and 1,2,4-triazino[4,3-*a*]quinoxalines as potential anticancer and antimicrobial hybrids, Med Chem Commun, 6 (2015) 202-211.
4. W. Combs, M. S. Rampulla, J. P. Demers, R. Falotico, J. B. Moore, Heteroatom analogues of Bemoradan: Chemistry and cardiotonic activity of l,4- benzothiazinyl-pyridazinones, J Med Chem, 35 (1991) 172-176.
5. Do¨ mling, A., 2006. Recent developments in isocyanide based multicomponent reactions in applied chemistry. Chem. Rev. 106, 17–89.
6. Dorsch, Dieter, Stieber, Frank, Schadt, Oliver, Blaukat, Andree, Inhibitors of tyrosine kinases, in particular Met kinase, 13/785471, 30 December, 2014.

7. Drummond, J., Johnson, G., Nickell, D.G., Ortwine, D.F., Bruns, R.F., Welbaum, B., 1989. Evaluation and synthesis of aminohydroxyisoxazoles and pyrazoles as potential glycine agonists. J. Med. Chem. 32, 2116–2128.
8. el-Shehry, M.F., Swellem, R.H., Abu-Bakr, Sh.M., el-Telbani, E.M., 2010. Synthesis and molluscicidal evaluation of some new pyrazole, isoxazole, pyridine, pyrimidine, 1,4-thiazine and 1,3,4-thiadiazine derivatives incorporating benzofuran moiety. Eur. J. Med. Chem. 45, 4783–4787.
9. Gokhan-Kelekc, N., Yabanogˇ lu, S., Ku¨ peli, E., Salgın, U., Ozgen, O., Ucar, G., Yesilada, E., Kendi, E., Yesiladaf, Y., Altan Bilgin, A., 2007. A new therapeutic approach in Alzheimer disease: some novel pyrazole derivatives as dual MAO-B inhibitors and anti-inflammatory analgesics. Bioorg. Med. Chem. 15, 5775–5786.
10. H. L. Qin, Z. P. Shang, I. Jantan, O. U. Tan, M. A. Hussain, M. Sherd, S. N. A. Bukhari, Molecular docking studies and biological evaluation of chalcone based pyrazolines as tyrosinase inhibitors and potential anticancer hybrids, RSC Adv, 5 (2015) 46330-46338.
11. Hejmadi and Momna, Introduction to cancer biology, Momna Hejmadi & Ventus Publishing ApS, ISBN 978-87-7681-478-6.
12. Koca, A. Ozgur, M. Er, M. Gumus, K. A. Coskun, Y. Tutar, Design and synthesis of pyrimidinyl acyl thioureas as novel Hsp90 inhibitors in invasive ductal breast cancer and its bone metastasis, Eur J Med Chem, 122 (2016) 280-290.
13. Kamal, A. V. S. Rao, M. V. P. S. Vishnuvardhan, T. S. Reddy, K. Swapna, C. Bagul, N. V. S. Reddya, V. Srinivasulu, Synthesis of 2-anilinopyridyletriazole hybrids as antimitotic hybrids, Org Biomol Chem, 13 (2015) 4879-4895.
14. J. Chen, J. Yan, J. Hu, Y. Pang, L. Huang, X. Li, Synthesis, biological evaluation and mechanism study of chalcone analogues as novel anti-cancer hybrids, RSC Adv, 5 (2015) 68128-76835.
15. J. Lebrun. S. Kulkarni, S. Crosignani, S. Crosignani, Synthesis of pyridazinone-amides derivatives, 20150376167, 31 December, 2015.
16. J. Ramirez-Prada, S. M. Robledo, I. D. Velez, M. D. P. Crespo, J. Quiroga, R. Abonia, A. Montoya, L. Svetaz, S. Zacchino, B. Insuasty, Synthesis of novel quinoline-based 4,5-dihydro-1*H*-pyrazoles as potential anticancer, antifungal, antibacterial and antiprotozoal agents, Eur J Med Chem, 131 (2017) 237-254.
17. K. Ochiai, S. Takita, A. Kojima, T. Eiraku, K. Iwase, T. Kishi, A. Ohinata, Y. Yageta, T. Yasue, D. R. Adams, Y. Kohno, Phosphodiesterase inhibitors. Part 5:

hybrid PDE3/4 inhibitors as dual bronchorelaxant/anti-inflammatory agents for inhaled administration, Bioorg Med Chem Lett, 23 (2013) 375-381.

18. K. Ochiai, S. Takita, T. Eiraku, A. Kojima, K. Iwase, T. Kishi, K. Fukuchi, T. Yasue, D. R. Adams, R. W. Allcock, Z. Jiang, Y. Kohno, Phosphodiesterase inhibitors. Part 3: Design, synthesis and structure-activity relationships of dual PDE3/4-inhibitory fused bicyclic heteroaromatic-dihydropyridazinones with anti-inflammatory and bronchodilatory activity, Bioorg Med Chem, 20 (2012) 1644- 1658.
19. K. Ozadali, F. Ozkanli, S. Jain, P. P. Rao, C. A. Velazquez-Martinez, Synthesis and biological evaluation of isoxazolo[4,5-*d*]pyridazin-4-(5*H*)-one analogues as potent anti-inflammatory agents, Bioorg Med Chem, 20 (2012) 2912-2922.
20. L. Beigelman, R. T. Hendricks, A. D. Stoycheva, J. Deval, S. K. Stevens, Process for the preparation of a pyridazinone derivative, WO 2015038660 A1, March 19, 2015.
21. M. Yamaguchi, K. Kamei, T. Koga, M. Akima, T. Kuroki, N. Ohi, Novel antiasthmatic agents with dual activities of thromboxane A2 synthetase inhibition and bronchodilation. 1. 2-[2-(1-Imidazolyl)alkyl]-1(2*H*)-phthalazinones, Concours Med, 25 (1993) 4052-4060.
22. N. Fukuda, Y. Ikemoto, Industrially advantageous method of producing compounds, US 20160002209, 07 January, 2016.
23. N. Kerru, P. Singh, N. Koorbanally, R. Raj, V. Kumar, Recent advances (2015-2016) in anticancer hybrids, Eur J Med Chem, (2017)
24. Padmaja, A., Payani, T., Reddy, G.D., Padmavathi, V., 2009. Synthesis, antimicrobial and antioxidant activities of substituted pyrazoles, isoxazoles, pyrimidine and thioxopyrimidine derivatives. Eur. J. Med. Chem. 44, 4557–4566.
25. Panda, S.S., Chowdary, P.V.R., Jayashree, B.S., 2009. Synthesis, antiinflammatory and antibacterial activity of novel indolyl-isoxazoles. Ind. J. Pharm. Sci. 71, 684–687.
26. Penning, T.D., Khilevich, A., Chen, B.B., Russell, M.A., Boys, M.L., Wang, Y., Duffin, T., Engleman, V.W., Finn, M.B., Freeman, S.K., Hanneke, M.L., Keene, J.L., Klover, J.A., Nickols, G.A., Nickols, M.A., Rader, R.K., Settle, S.L., Shannon, K.E., Steininger, C.N., Westlin, M.M., Westlin, W.F., 2006. Synthesis of pyrazoles and isoxazoles as potent alpha(v)beta3 receptor antagonists. Bioorg. Med. Chem. Lett. 16, 3156–3161.
27. Rakesh Tiwle “An Exhaustive Review On Solubility Enhancement For Hydrophobic Compounds By Possible Applications Of Novel Techniques.” Science Alert –Trends

Research In Applied Science And Research. 7(8): 596-619; 2012.

28. R. F. George, M. A. Fouad, I. E. O. Gomaa, Synthesis and cytotoxic activities of some pyrazoline derivatives bearing phenyl pyridazine core as new apoptosis inducers, Eur J Med Chem, 112 (2016) 48-59.
29. R. M. Burger, Cleavage of nucleic acids by bleomycin, Chem Rev, 98 (1998) 1153-1170.
30. R. Zhao, I. D. Goldman, The proton-coupled folate transporter: physiological and pharmacological roles, Curr Opin Pharmacol, 13 (2013) 875-880.
31. Rusconi, S., Moonis, M., Merrill, D.P., Pallai, P.V., Neidhardt, E.A., Singh, S.K., Osburne, M.S., Profy, A.T., Jenson, J.C., Hirsch, M.S., 1996. Naphthalene sulfonate polymers with CD4-blocking and anti-human immunodeficiency virus type 1 activities. Antimicrob. Agents Chemother. 40, 234–236.
32. S. Mogilski, M. Kubacka, A. Redzicka, G. Kazek, M. Dudek, W. Malinka, B. Filipek, Antinociceptive, anti-inflammatory and smooth muscle relaxant activities of the pyrrolo[3,4-*d*]pyridazinone derivatives: Possible mechanisms of action, Pharmacol Biochem Behave, 133 (2015) 99-110.
33. Rakesh Tiwle, Effect of PH of AlCl3 Solution on Drug Entrapment Efficiency of IPN Beads, International Journal of Chemistry and Pharmaceutical Sciences, IJCPS, 2014, Vol.2(6): 878-881.
34. Selby, T. Paul, Deprez, N. Ryan, Stevenson, T. Martin, Taggi, A. Edmund, Debergh, J. Robbins, Pyridazinone herbicides, WO/2015/168010, 5 November, 2015.
35. Selvam, C., Jachak, S.M., Thilagavathi, R., Chakraborti, A.K., 2005. Design, synthesis, biological evaluation and molecular docking of curcumin analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents. Bioorg. Med. Chem. Lett. 15, 1793– 1797.
36. T. Fusaka, Process for the preparation pyridazinone compound and use, WO2010104217 A1, 16 September, 2010.