Advances in Bioresearch
Adv. Biores., Vol 14 (5) September 2023: 147-152
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.5.147152

Advances in Bioresearch

ORIGINAL ARTICLE

Synthesis, Characterization, and Biological evaluation of α-cyano substituted Chalcones as anti-breast Cancer and Anti-inflammatory agents

Pravin S. Bhale,[a] Dnyaneshwar M. Sirsat,[b] Samadhan A. Shenmare[c], Sadanand N. Shringare[d]
[a]Department of Chemistry, Yeshwantrao Chavan Mahavidyalaya, Tuljapur, Dist-Osmanabad-413 601, Maharashtra, India.
[b]Department of Chemistry, Anandibai Raorane A. C. S. College, Vaibhavwadi, Dist-Sindhudurg-416 810, Maharashtra, India.
[c]Department of Chemistry, Arts, Science and Commerce College, Naldurg, Dist-Osmanabad-413 602, Maharashtra, India.
[d]School of Chemical Sciences, P. A. H. Solapur University Solapur-413 255, Maharashtra, India.
Address for correspondence: Email-sadanandshringare@gmail.com

ABSTRACT
*A series of α-cyano substituted chalcones were synthesized and evaluated for their anticancer and anti-inflammatory activity. Adequate confirmation of the synthesized compounds was achieved using modern analytical techniques like FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS spectroscopic techniques. Nine compounds were tested against anti-cancer cell line, MCF-7 and calculated GI_{50} values from averages of 3 experiments. Compound **3d** exhibited potent activity (GI_{50} = 25.4 μM) against the MCF-7 cell line which was almost as good as that of standard drug adriamycin. Synthesized compounds were also evaluated for their anti-inflammatory activity. Most of the α-cyano substituted chalcones displayed significant anti-inflammatory activity.*
KEYWORDS: *Indole, Chalcone, Anti-cancer activity, Anti-inflammatory activity*

Received 11.05.2023 Revised 21.06.2023 Accepted 25.07.2023

How to cite this article:
Pravin S. Bhale, Dnyaneshwar M. Sirsat, Samadhan A. Shenmare, Sadanand N. Shringare .Synthesis, characterization, and biological evaluation of α-cyano substituted chalcones as anti-breast cancer and anti-inflammatory agents. Adv. Biores. Vol 14 [5] September. 2023. 147-152.

INTRODUCTION:

Cancer continues to be the leading cause of death worldwide, with an increasing number of people being diagnosed and dying from it each year. Various etiological aspects contribute to the development of cancer, but one of the mainly significant is the modernization of our society. [1,2,3]. As per the statistics declared by the National Centre for Health Statistics (NCHS) in the year 2022, approximately 19.8 million patients affected by cancer were authenticated and about 0.61 million cancer casualties were recorded considering the global scenario.

At the present time, chemotherapy is the greatest approach used for the treatment of cancer over radiation therapy [4]. It is frequently used in combination with other cancer treatments such as surgery, radiation therapy, and immunotherapy. Although it can be efficient in killing cancer cells, it can also cause fatigue, nausea, hair loss, and an increased risk of infection. Nevertheless, chemotherapy remains a significant part of cancer treatment to progress the efficiency of treatment and amplify the probability of successful outcomes. Because of the occurrence of drug resistance, patient survival rates are still left not good enough. This strictly impedes patient prognosis growth. Hence there is continuously an ongoing need to discover new anti-cancer agents to deal with the drawbacks of the existing chemotherapeutic treatments. In order to efficiently target malignant cells while sparing healthy cells, medicinal chemists are presently working to make targeted antitumor drugs with high effectiveness and less toxicity [5-10]. In this regard, indole offers a brilliant scaffold that can be tailored for favorable biological activity. Indolyl chalcones have

received remarkable attention because of their broad range of biological activities, such as antioxidant, antibacterial, anti-fungal, anticancer, and anti-diabetic activities [11]. El-Sawy *et.al.* reported N-methyl sulphonyl and N-benzenesulphonyl-3-indolyl heterocycles as anticancer and antimicrobial agents [12]. Kumar *et.al.* also reported α-cyano bis(indolyl)chalcones as novel anticancer agents [13]. Recently, our research group reported α-cyano substituted bis-indolyl chalcone [14], extended conjugated indolyl chalcones [15], E)-3-(benzo[d]thiazol-2-ylamino)-2-(1-methyl-1H-indole-3-carbonyl)-3(methylthio) acrylonitrile derivatives [16], 1,3,4,5-tetrasubstituted pyrazole derivatives [17], indole-pyrazole based α-cyano substituted chalcones [18] and indolyl bis-chalcone [19] as potent anti-breast cancer agents. In our constant efforts to discover potent anticancer agents [20-22], herein we have synthesized a series of α-cyano substituted chalcones and *in vitro* evaluated for their anti-breast cancer and anti-inflammatory activity.

MATERIAL AND METHODS

All the chemicals used for the synthesis of α-cyano substituted chalcones were of synthetic grade and obtained from commercial sources. The development of the reactions was checked by thin-layer chromatography (TLC) using TLC plate (silica gel 60 F254, aluminium back, Merck) with visualization by UV light. Melting points of α-cyano substituted chalcones were determined by the open-end capillary method and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as internal standard and chemical shifts are reported in δ units and the coupling constants (J) are reported in Hertz. IR spectra were recorded on FT-IR Nicolet iS 10 spectrophotometer in KBr pellet and Mass spectra were obtained with a Shimadzu LCMS-2010EV. Anticancer activities were carried out under the supervision of Dr. Jyoti Kode, Scientific Officer, Tata Memorial Centre, and Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai-410210.

General procedure for the synthesis of α-cyano substituted chalcones (3a-i)

To a mixture of 3-(1*H*-indol-3-yl)-3-oxopropane nitrile **1a-c** (1 mmol) in ethanol (10 mL) was added piperidine (0.3 mL) and stirred for 15 min. Then, added the heterocyclic aldehydes **2a-c** (1 mmol) and this mixture was heated at reflux for 1-4 h. After completion of the reaction (monitored by TLC), the reaction mixture was poured over crushed ice and acidified with acetic acid. The precipitated solid was filtered, washed with water, and oven-dried. It was column purified by column chromatography using silica gel mesh size, 100–200, and elution with 10% ethyl acetate in pet ether.

Spectral data of representative compounds

(E)-2-(1H-indole-3-carbonyl)-3-(4-methylthiazol-5-yl)acrylonitrile (3a): Yellow solid; 94%; 270–272^{o}C; IR (cm^{-1}): 3285 (NH), 2228 (CN), 1676 (C=O), 1618 (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): □ = 10.91 (broad s, 1H, NH), 9.23 (s, 1H), 8.42 (s, 1H), 8.19 (s, 1H, C=CH), 7.99-7.56 (m, 3H), 7.01 (d, *J* = 6.8Hz, 1H), 2.33 (s, 3H, CH_3); HRMS: Calculated for $C_{16}H_{11}N_3OS$; Exact mass: 293.0611, found: 294.1032 (ESI M+H).

(E)-2-(1H-indole-3-carbonyl)-3-(pyridine-3-yl)acrylonitrile (3b): Yellow solid; 88%; 246–248^{o}C; IR (cm^{-1}): 3234 (NH), 2222 (CN), 1628 (C=O), 1603 (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): □ = 10.99 (broad s, 1H, NH), 8.69 (s, 1H), 8.39 (s, 1H), 8.31-8.15 (m, 3H), 7.96 (d, *J* = 7.0 Hz, 1H), 7.55-7.29 (m, 3H), 7.03 (d, *J* = 6.4 Hz, 1H); HRMS: Calculated for $C_{17}H_{11}N_3O$; Exact mass: 273.0905, found: 274.1108 (ESI M+H).

(E)-2-(5-bromo-1H-indole-3-carbonyl)-3-(4-methylthiazol-5-yl)acrylonitrile (3d): Yellow solid; 92%; 260–262^{o}C; IR (cm^{-1}): 3265 (NH), 2223 (CN), 1667 (C=O), 1600 (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): □ = 11.00 (broad s, 1H, NH), 9.06 (s, 1H), 8.35 (s, 1H), 8.01 (s, 1H, C=CH), 7.64-7.40 (m, 3H), 2.45 (s, 3H, CH_3); HRMS: Calculated for $C_{16}H_{10}BrN_3OS$; Exact mass: 370.9702, found: 371.8803 (ESI M+H).

(E)-3-(benzo[d][1,3]dioxol-5-yl)-2-(5-methoxy-1H-indole-3-carbonyl)acrylonitrile (3i): Yellow solid; 90%; 242–244^{o}C; IR (cm^{-1}): 3243 (NH), 2212 (CN), 1688 (C=O), 1615 (C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): □ = 11.32 (broad s, 1H, NH), 8.62 (s, 1H), 7.72 (s, 1H), 7.45 (s, 1H, C=CH), 7.30 (d, *J* = 6.1Hz, 1H), 7.20-6.99 (m, 3H), 6.70 (d, *J* = 6.1Hz, 1H), 6.02 (s, 2H, -CH_2-), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): □ = 186.43, 154.34, 153.91, 148.05, 147.90, 138.32, 129.40, 128.11, 127.07, 122.55, 115.76, 112.19, 112.01, 111.08, 109.00, 108.50, 104.75, 104.21, 101.10, 55.78; HRMS: Calculated for $C_{20}H_{14}N_2O_4$; Exact mass: 346.1011, found: 347.2105 (ESI M+H).

The procedure of the SRB-assay: Tumor cells (human breast cancer cell line MCF-7) were grown in tissue culture flasks in a growth medium (RPMI-1640 with 2 mM glutamine, pH 7.4, 10% fetal calf serum, 100 □g/mL streptomycin, and 100 units/mL penicillin) at 37^{o}C under the atmosphere of 5% CO_2 and 95% relative humidity employing a CO_2 incubator. The cells at the subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and placed in a growth medium. The cells with more than 97% viability (trypan blue exclusion) were used for cytotoxicity studies. An aliquot of 100 □L of cells was transferred to a well of the 96-well tissue culture plate. As mentioned

above, the cells were allowed to grow for one day at 37°C in a CO_2 incubator. The test materials at different concentrations were then added to the wells and cells were further allowed to grow for another 48 h. Suitable blanks and positive controls were also included. Each test was performed in triplicate. The cell growth was stopped by gently layering 50 µL of 50% trichloroacetic acid. The plates were incubated at 4°C for an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were gently pipetted out and discarded. The plates were washed five times with doubly distilled water to remove TCA, growth medium, etc, and were air-dried. 100 µL of SRB solution (0.4% in 1% acetic acid) was added to each well and the plates were incubated at ambient temperature for half an hour. The unbound SRB was quickly removed by washing the wells five times with 1% acetic acid. Plates were air dried, tris-buffer (100 µL of 0.01 M, pH 10.4) was added to all the wells, and plates were gently stirred for 5 min on a mechanical stirrer. The optical density was measured on an ELISA reader at 540 nm. The cell growth in the absence of any test material was considered 100% and in turn growth inhibition was calculated. GI_{50} values were determined by regression analysis.

***In vitro, anti-inflammatory activity by protein denaturation method*:** The reaction mixture (10 ml) consisted of 0.4 ml of egg albumin (from fresh hen's egg), 5.6 ml of phosphate-buffered saline (PBS, pH 6.4), and 4 ml of synthetic derivative (1 mM). A similar volume of double-distilled water served as a control. Then the mixtures were incubated at (37°C ± 2) in an incubator for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm by using a vehicle as blank. Diclofenac sodium (1 mM) was used as a reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

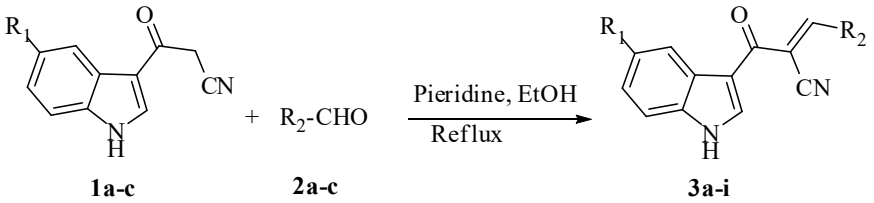
% inhibition = 100 x (Vt / Vc - 1)

Where, Vt = absorbance of a test sample, Vc = absorbance of control.

RESULTS AND DISCUSSION

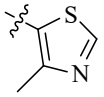
Chemistry

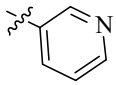
In the recent study, syntheses of α-cyano substituted chalcones (**3a-i**) were accomplished by the Knoevenagel condensation of substituted 3-cyanoacetyl indoles **1a-c** with substituted heterocyclic aldehyde **2a-c** in the presence of piperidine in ethanol (**Scheme 1**). The starting compounds for the synthesis of title compounds, namely 3-cyanoacetyl indoles **1a-c** synthesized in good yields from the reaction of substituted indoles with cyanoacetic acid in the presence of acetic anhydride using the method described in the literature with minor modifications [23]. The target molecules obtained were purified by column chromatography using silica gel (mesh size, 100–200) and elution with 10% ethyl acetate in pet ether. The structures of newly synthesized α-cyano substituted chalcones (**3a-i**) were confirmed with IR, ^{1}H NMR, ^{13}C NMR, and HRMS techniques.

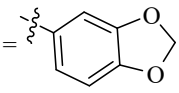


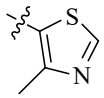
1a-c **2a-c** **3a-i**

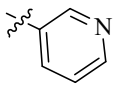
Where,

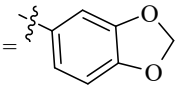
3a R_1 = H, R_2 = 

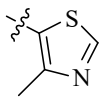
3b R_1 = H, R_2 = 

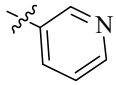
3c R_1 = H, R_2 = 

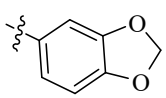
3d R_1 = Br, R_2 = 

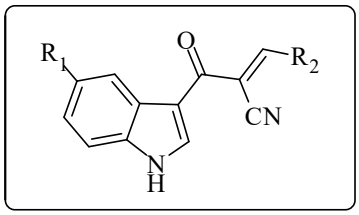
3e R_1 = Br, R_2 = 

3f R_1 = Br, R_2 = 

3g R_1 = OCH_3, R_2 = 

3h R_1 = OCH_3, R_2 = 

3i R_1 = OCH_3, R_2 = 



Scheme 1: Synthesis of α-cyano substituted chalcones
Reagents and condition: a) Piperidine, Ethanol, Reflux 1-4 h

Anticancer activity

All the newly synthesized α-cyano substituted chalcones (**3a-i**) were screened for their *in vitro* anticancer potencies in human breast cancer cell line MCF by using the sulforhodamine B (SRB) assay method [24]. Adriamycin, a most effective anticancer drug used as the reference standard. Three parameters namely growth inhibition (GI_{50}), total growth inhibition (TGI), and lethal concentration (LC_{50}) were estimated during the screening process and the results are presented in **Table 1**.

Table 1.*In vitro* cytotoxicity screening of α-cyano substituted chalcones (**3a-i**).[a]

Compound	MCF-7		
	LC_{50}[b]	TGI[c]	GI_{50}[d]
3a	>100	>100	45.1
3b	>100	>100	>100
3c	>100	>100	65.3
3d	>100	40.0	25.4
3e	>100	>100	>100
3f	>100	>100	63.8
3g	>100	>100	36.5
3h	>100	>100	>100
3i	>100	61.3	40.2
Adriamycin	>100	9.0	<0.1

[a] Concentrations in μM; [b]Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) calculated from $[(Ti - Tz)/Tz]$ x100 = -50; [c]Drug concentration resulting in total growth inhibition (TGI) will calculated from $Ti = Tz$; [d]Growth inhibition of 50% (GI_{50}) calculated from $[(Ti - Tz)/(C - Tz)]$ x 100 = 50

It is worth mentioning that most of the compounds were noticeably cytotoxic against MCF-7 breast cancer cell line compared to adriamycin a standard reference drug with the concentration of the drug that produced 50% inhibition of cell growth (GI_{50}). Among the compounds screened, compound **3d** exhibited potent activity (GI_{50} = 25.4 μM) against the MCF-7 cell line. Compounds **3a, 3c, 3f, 3g,** and **3i** exhibited good cytotoxicity in the range of 36.5 μM to 65.3 μM. On the other hand, compounds **3b, 3e** and **3h** showed weak cytotoxicity (GI_{50} = >100 μM**)** against the MCF-7 cell line.

In a comparison of the TGI concentrations, compound **3d** exhibited significant inhibition (TGI = 40.0 μM) as compared to the standard drug adriamycin (TGI = 9.0 μM) however compounds **3i** exhibited moderate activity (TGI = 61.3 μM) against the MCF-7 cell line. All other compounds were found inactive (TGI>100 μM) as compared to standard drugs.

The LC_{50} concentrations of the compounds were compared to that of adriamycin to get an insight into the cytotoxic effects of these compounds against the MCF-7 cell line. The compounds were inactive (LC_{50}>100 μM) like adriamycin (LC_{50} = >100 μM) against the MCF-7 cell line.

***In vitro* anti-inflammatory activity**

Inhibition of albumin denaturation

The denaturation of proteins is a well-documented cause of inflammation. In the current study, the *in vitro* anti-inflammatory effect of α-cyano substituted chalcones (**3a-i**) was evaluated against the denaturation of egg albumin and obtained results are summarized in **Table 2**.

Table 2. *In vitro* anti-inflammatory α-cyano substituted chalcones **(3a-i)**

Compound	Anti-inflammatory activity
	% inhibition (1mM)
3a	79.98
3b	49.74
3c	64.32
3d	42.58
3e	54.68
3f	61.17
3g	77.22
3h	51.74
3i	60.34
Diclofenac sodium	90.21

Compounds **3a** and **3g** showed significant inhibition (79.98 and 77.22% respectively) compared to the Diclofenac sodium, a standard anti-inflammation drug (90.21%) at 1 mM concentration. All the other compounds showed weak to moderate inhibition (42.58 - 64.32%) as compared to the Diclofenac sodium.

CONCLUSION

In conclusion, we synthesized -cyano-substituted chalcones by Knoevenagel condensation and *in vitro* evaluated them for their cytotoxic potential against breast carcinoma (MCF-7 cells) and inhibition of egg albumin denaturation. Most of the synthesized compounds exhibited significant antitumor activities. Among them, compound **3d** exhibited higher activity against breast carcinoma as good as adriamycin. Most of the α-cyano substituted chalcones displayed significant anti-inflammatory activity. The present investigation has thus offered the impetus for the design and development of more potent anticancer leads.

REFERENCES

1. Sung, H., Ferlay, J., Siegel, R., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71(3): 209-249.
2. Ganesh, K., Massagué, J.(2021). Targeting metastatic cancer. Nat. Med., 27(1): 34-44.
3. Merriel, S., Ingle, S., May, M., Martin, R.(2021). Retrospective cohort study evaluating clinical, biochemical and pharmacological prognostic factors for prostate cancer progression using primary care data. BMJ Open, 11(2): 1-8, e044420.
4. El-Hussein, A,, Manoto, S., Ombinda-Lemboumba, S., Alrowaili, Z., Mthunzi-Kufa, P. (2021). A review of chemotherapy and photodynamic therapy for lung cancer treatment. Anticancer Agents Med. Chem. 21(2): 149-161.
5. Charmsaz, S., Collins, D., Perry, A., Prencipe, M., (2019). Novel strategies for cancer treatment: highlights from the 55th IACR annual conference. Cancers. 11(8): 1125.
6. Arruebo, M., Vilaboa, N., Sáez-Gutierrez, B., Lambea, J., Tres, A., Valladares, M., González-Fernández, A. (2011). Assessment of the evolution of cancer treatment therapies. Cancers. 3(3): 3279-3330.
7. Moses, M., Brem, H., Langer, R. (2003). Advancing the field of drug delivery: taking aim at cancer. Cancer Cell. 4(5): 337-341.
8. Shapira, A., Livney, Y., Broxterman, H., Assaraf, Y. (2011). Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance. Drug Resist. Updat. 14(3): 150-163.
9. Mondal, J., Panigrahi, A., Khuda-Bukhsh, A. (2014). Conventional chemotherapy: problems and scope for combined therapies with certain herbal products and dietary supplements. Austin J. Mol. Cell Biol. 1(1): 1-10.
10. El-Readi, M., Althubiti, M. (2019). Cancer nanomedicine: a new era of successful targeted therapy. J. Nanomater. 2019: 1-13.
11. Seo, W., Kim, J., Kang, J., Ryu, H., Curtis-Long, M., Lee, H., Yang, M.,Park, K. (2005). Sulfonamide chalcone as a new class of α-glucosidase inhibitors, Bioorganic & Medicinal Chemistry Letters. 15 (24): 5514–5516.
12. El-Sawy, E., Mandour, A., M.El-Hallouty, S., Shaker, K. and MohamedAbo-Salem, H. (2013). Synthesis, antimicrobial and anticancer activities of some new N-methylsulphonyl and N-benzenesulphonyl-3-indolyl heterocycles: 1st Cancer Update. Arabian Journal of Chemistry. 6(1): 67-78.

13. Kumar, D. Kumar, N., Tantak, M., Ogura, M., Kusaka, E. and Ito T. (2014). Synthesis and identification of α-cyano bis(indolyl)chalcones as novel anticancer agents. Bioorganic & Medicinal Chemistry Letters. 24(22):5170-5174.
14. Bhale, P., Chavan, H., Dongare, S., Shringare, S., Mule, Y., Choudhari, P., and Bandgar, B. (2018). Synthesis, Characterization and Evaluation of 1,3-Bisindolyl-2-Propen-1- One Derivatives as Potent Anti-Breast Cancer Agents. Current Bioactive Compounds. 14(3): 299-308.
15. Bhale, P., Chavan, H., Dongare, S., Shringare, S., Mule, Y., Nagane, S. and Bandgar, B. (2017). Synthesis of extended conjugated indolyl chalcones as potent anti-breast cancer, anti-inflammatory and antioxidant agents. Bioorganic & Medicinal Chemistry Letters. 27(7):1502-1507.
16. Bhale, P., Chavan, H., Dongare, S., Sankpal, S. and Bandgar, B. (2018). α-Aroylketene Dithioacetal Mediated Synthesis of (E)-3-(benzo[d]thiazol-2-ylamino)-2-(1-methyl-1H-indole-3-carbonyl)-3-(methylthio)acrylonitrile Derivatives and their Biological Evaluation. Anti-Cancer Agents in Medicinal Chemistry. 18(5): 757-764.
17. Bhale, P., Bandgar, B., Dongare, S., Shringare, S., Sirsat, D. and Chavan, H. (2019). Ketene dithioacetal mediated synthesis of 1, 3, 4, 5-tetrasubstituted pyrazole derivatives and their biological evaluation. Phosphorus, Sulfur, and Silicon and the Related Elements. 194(8): 843-849.
18. Bhale, P., Shringare, S., Khade, A. and Chavan, H. (2021). Synthesis, Characterization and Biological Evaluation of Indole-Pyrazole Amalgamated α-Cyano Substituted Chalcones. Anti-Cancer Agents in Medicinal Chemistry. 21(16): 2216-2223.
19. Bhale, P., Chavan, H., Endait, R., Kadam, A., Bopalkar, R. and Gaikwad, M.(2021). Synthesis and biological evaluation of bis-chalcone as anti-breast cancer and anti-oxidant agents. Croatica Chemica Acta. 94(1):35-41.
20. Dongare, S., Bandgar, B., Bhale, P., Shringare, S., Chavan, H. (2019). Design, Synthesis, and Spectroscopic Study of 7-Azaindolyl Hydrazones with Anti-Breast Cancer Activity, Croatica Chemica Acta. 92(1): 1-9.
21. Shringare, S., Chavan, H., Bhale, P., Dongare, S., Mule, Y. Patil, S. and Bandgar, B. (2018). Synthesis and pharmacological evaluation of combretastatin-A4 analogs of pyrazoline and pyridine derivatives as anticancer, anti-inflammatory and antioxidant agents. Medicinal Chemistry Research. 27:1226-1237.
22. Shringare, S., Chavan, H., Bhale, P., Dongare, S., Mule, Y., Kolekar, N. and Bandgar, B.(2018). Synthesis and pharmacological evaluation of pyrazoline and pyrimidine analogs of combretastatin-A4 as anticancer, anti-inflammatory and antioxidant agents. Croatica Chemica Acta. 91(3):357–366.
23. Slatt, J.; Romero, I.; Bergman, J.(2004). Cyanoacetylation of Indoles, Pyrroles and Aromatic Amines with the Combination Cyanoacetic Acid and Acetic Anhydride. Synthesis. 16:2760.
24. Skehan, P., Strong, R., Scadiaro, D., Monks, A., Mc-Mahon, J., Vistica, D., Warren, J., Bokesch, H., Kenney, S. and Boyed, M.(1990). New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. Journal of the National Cancer Institute. 82(13):1107-1112.