INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [Impact Factor: 7.581]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 9, Issue - 12, December - 2023 Publication Date: 31/12/2023

DOIs:10.2015/IJIRMF/202312015 --:-- Research Paper / Article / Review

Antioxidant and anti-inflammatory potential of newer fused 2,3-disubstituted benzopyrimidines

[1]Vachala S. Dinakaran, [2]Srinivasan K. Kothari
[1]Professor and HOD, Department of Pharmaceutical Chemistry, RR College of Pharmacy, **RR** Institutions, Chikkabanavara, Bangalore-560 090, Karnataka, India.
[2]Professor, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576 104, Udupi District, Karnataka, India.
Email: [1]sdvachala@gmail.com, [2]pansrini@yahoo.co.in

***Abstract*:** *Bicyclic aromatic heterocycles such as benzo pyrimidines were well-known pharmacophores in the drug discovery process. These privileged structures were well studied and represented in chemical compound libraries. In pursuit of further investigations in support of these findings, this heterocyclic scaffold, benzopyrimidin-4-ones have been synthesized and screened for their biological properties. Condensation of 2-amino benzoic acid with 4-fluoro benzoyl chloride resulted in N-acyl substituted-2-amino benzoic acid. This on cyclization with various aromatic and aliphatic primary amines yielded the respective 2, 3-disubstituted benzopyrimidin-4-ones (BP 1-6). The completion of the reaction was monitored by Thin layer chromatography. The structure of the synthesized compounds was confirmed by spectral studies. All these compounds were screened for their in vitro antioxidant, in vivo acute toxicity and anti-inflammatory activities. Antioxidant and anti-inflammatory potentials of the compounds were tested by DPPH radical scavenging and carrageenan induced paw oedema method in rats respectively. Acute toxicity study was carried out by up and down stair case method. Acute toxicity study results revealed the absence of toxic manifestations at a dose of 1000 mg/kg body weight. Thus, the compounds were considered safe. One tenth of the safer dose was selected for the in vivo anti-inflammatory activity. All the tested compounds showed good percentage of free radical scavenging and anti-inflammatory activities. To conclude the present work, the presence of electron withdrawing substituents such as, 4-chlorophenyl, 3-chloro-4-fluorophenyl and N,N-dimethylaminoethyl functions at the 3rd position and the electronegative 4-fluoro substituted phenyl ring on 2nd position played a vital role in improving the biological profile of the scaffold.*

Keywords: *Benzopyrimidin-4-ones, Antioxidant. Anti-inflammatory, Acute toxicity, DPPH radical scavenging, Rat paw oedema.*

1. INTRODUCTION:

The chemistry of heterocyclic compounds has been a thought-provoking field of study for a longer time. Among them, various substituted benzimidazoles are the most privileged heterocycles for medicinal chemists owed to their broad spectrum of biological activities. The synthesis of novel fused benzo-4-pyrimidinone derivatives and investigation of their chemical and biological behaviours has gained more importance in recent decades in view of their pharmacological activities such as anti-inflammatory (1-2), analgesic (3), antiulcer (4), antihelminthic (5), antihepatitis (6), antifungal (7), MAO inhibitory activities (8), anticonvulsant (9), antitumor (10-11), antiprotozoal (12), antibacterial (13) and antiproliferative activities (14), as well as inhibitory effects for thymidylate synthase and poly-(ADP-ribose) polymerase (PARP) activities (15). Furthermore, the 2-substituted benzopyrimidinones in particular have been utilized as peptidomimetic scaffolds with specificity for cholecystokinin, angiotensin and certain cell adhesion receptors (16). Several 2,3-disubstituted benzopyrimidine derivatives were synthesized and tested for different biological activities. The reports showed that the aryl substitution at 3rd position enhances the biological activity. Several methods have been reported for the synthesis of benzopyrimidin-4-one derivatives (17). However, these methods suffer from drawbacks, such as longer reaction time, complicated workup, and use of expensive and hazardous chemicals with low yield (18). Usage of microwave irradiation (MWI) is well known for the synthesis of a variety of compounds wherein chemical reactions are accelerated because of selective absorption of microwave by polar molecules. The coupling of MWI with solid-supported reagents under solvent-free conditions provides unique chemical processes with special attribute, such as enhanced reaction rate, higher yield and greater selectivity. However, this technique requires an appreciable amount

of solvent for adsorption of reactants and elution of products. In view of ongoing research on neat synthesis, when the solvent-free reactions are coupled with MWI, the same proved to be advantageous considering environmental reasons as well as due to their uniform heating effect and shorter reaction times (19). A combination of two or more biologically active moiety could increase or decrease the activity (20). Keeping this aspect in mind, in view of the involvement of biological activities and the eco-friendly synthesis of benzopyrimidin-4-one, the purpose of the present study is to construct some novel 2-para chlorophenyl-4-substitutued-benzopyrimidin-4-ones as potential antioxidant and anti-inflammatory agents.

2. MATERIALS AND METHODS:

EXPERIMENTAL

CHEMISTRY

The chemicals used in the study were listed in Table 1. The melting point of the synthesized compounds was determined in open capillary tubes and was uncorrected. The IR spectra of the compounds were recorded in the range of 500–4,000 cm^{-1} on Shimadzu FT-IR 8310 using KBr pellets. The ^{1}H-NMR spectra were recorded on Joel, model GSV-400 MHz spectrometer using$CDCl_3$/DMSO-d_6 as solvent. The chemical shifts were reported as parts per million downfield from tetramethylsilane (Me_4Si). Mass spectra were recorded on the Shimadzu GC-MS QP5050. The reactions were monitored by thin layer chromatographic analysis on precoated SiO_2 gel plates. The spots were visualized by UV light. Micro analyses for C, H and N were performed in Heraeus CHN Rapid Analyser. Satisfactory C, H and N analyses were obtained for all the compounds.

GENERAL METHOD FOR SYNTHESIS OF FUSED 2,3-DISUBSTITUTED BENZOPYRIMIDIN-4-ONES (BP 1-6):

Step 1. Preparation of N-acyl anthranilic acid

N-acyl anthranilic acid derivatives were synthesized by Schotten-Baumann reaction (21). In an iodine flask, 0.5 g of anthranilic acid was placed with 5 mL of 10 % sodium hydroxide solution. Then, 1 mL of 4-fluoro benzoyl chloride was added slowly with constant mixing in between of each addition. During the additions, the flask was kept in an ice bath. Finally, the flask was shaken vigorously for 5-10 min. The content of the flask was then, poured into cold water with stirring and neutralized with dilute Hydrochloric acid. The separated solid was filtered, washed with water and recrystallized from ethanol as colorless crystalline solid.

Step 2. Synthesis of 2,3-disubstituted benzopyrimidin-4-ones (BP 1-28)

Synthesis of 2,3-disubstituted benzopyrimidin-4-one derivatives were carried out according to the published procedure (22). Equimolar amount of the prepared *N*-acyl anthranilic acid was condensed with various primary amines in the presence of dicyclohexylcarbodiimide (DCC) in a 250 mL Erlenmeyer flask. The reaction mixture was microwave irradiated for 4-14 min, and cooled. Ice cold water was then added and the solid separated was filtered off, washed with cold ethanol and recrystallized from ethanol as fine needles.

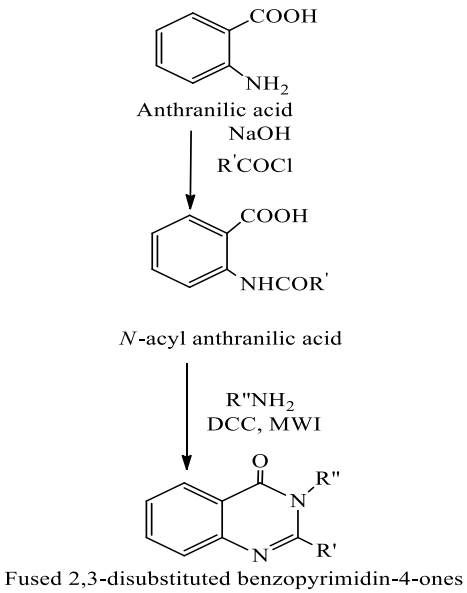


Figure 1. Synthesis of fused 2,3-disubstituted benzopyrimidin-4-ones (BP 1-6)

Table 1. List of chemicals used

S.No	Chemicals Name	Manufacturer / Supplier
1.	4-fluorobenzoyl chloride	Sigma Aldrich, Germany
2.	Sodium hydroxide	Sigma Aldrich, Germany
3.	DPPH• reagent	Sigma Aldrich, Germany
4.	Anthranilic acid	Sigma Aldrich, Germany
5.	Methanol	Merk Ltd, Mumbai
6.	Dimethylsulfoxide	Sigma Aldrich, Germany
7.	Ethanol	Sigma Aldrich, Germany
8.	*N,N*-dimethylethylenediamine	Sigma Aldrich, Germany
9.	4-chloroaniline	Sigma Aldrich, Germany
10.	3-chloroaniline	Sigma Aldrich, Germany
11.	4-chlorobenzylamine	Sigma Aldrich, Germany
12.	3-chloro-4-fluoroaniline	Sigma Aldrich, Germany
13.	4-fluorobenzylamine	Sigma Aldrich, Germany
14.	Dicyclohexylcarbodiimide	Sigma Aldrich, Germany
15.	Ascorbic acid	SD fine chemicals, Mumbai
16.	Ibuprofen	SD fine chemicals, Mumbai

Table 2. List of equipment or instrument used

S.No	Domestic Microwave synthesizer	Catalyst, CATA2R
1	Melting point apparatus	Shital scientific industries, Mumbai
2	Magnetic stirrer	Spinit
3	FT-IR 8310 Spectrophotometer	Shimadzu, Japan
4	FT NMR Spectrometer- AVANCE III	Bruker, Germany
5	GC-MS Spectrometer-Jeol GCMATE II	Tokyo, Japan
6	Microtitre plate reader	Biotek, USA
7	Digital plethysmometer	Ugo Basile Company, Italy

Table 3. Codes of the synthesized benzopyrimidin-4-ones along with their substituents

Compound code	R′	R″
BP-1	4-fluorophenyl	3-chloro-4-fluorophenyl
BP-2	4-fluorophenyl	4-fluorobenzyl
BP-3	4-fluorophenyl	4-chlorobenzyl
BP-4	4-fluorophenyl	4-chlorophenyl
BP-5	4-fluorophenyl	3-chlorophenyl
BP-6	4-fluorophenyl	2-(dimethylamino)ethyl

Table 4. IUPAC name of the synthesized 2,3-disubstituted benzopyrimidin-4-ones

Code	**Molecular formula**	**IUPAC name**
BP-1	$C_{20}H_{11}ClF_2N_2O$	3-(3-chloro-4-fluorophenyl)-2-(4-fluorophenyl) benzopyrimidin-4-one
BP-2	$C_{21}H_{14}F_2N_2O$	3-(4-fluorobenzyl)-2-(4-fluorophenyl)benzopyrimidin-4-one
BP-3	$C_{21}H_{14}ClFN_2O$	3-(4-chlorobenzyl)-2-(4-fluorophenyl)benzopyrimidin-4-one
BP-4	$C_{20}H_{12}ClFN_2O$	3-(4-chlorophenyl)-2-(4-fluorophenyl)benzopyrimidin-4-one
BP-5	$C_{20}H_{12}ClFN_2O$	3-(3-chlorophenyl)-2-(4-fluorophenyl)benzopyrimidin-4-one
BP-6	$C_{18}H_{18}FN_3O$	3-(2-(dimethylamino)ethyl)-2-(4-fluorophenyl) benzopyrimidin-4-one

BIOLOGICAL SCREENING

In vitro antioxidant activity

Diphenylpicrylhydrazyl radical (DPPH radical) scavenging assay was the simple, effective and convenient method to determine the free radical scavenging potential of a compound. DPPH radical solution was prepared by dissolving 3.96 mg of DPPH reagent in 50 mL of methanol. Stock solutions of 2000 μg/mL of the synthesized compounds and the standard, ascorbic acid were prepared individually using methanol. These solutions were serially diluted with the same solvent to obtain the required concentrations (1000 μg/mL to 31.25 μg/mL). The antioxidant activity of the test compounds and the standard was assessed using 96 well microtitre plate. To 100 μL of DPPH radical solution, 100 μL of the test sample or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 $^{\circ}$C for 20 min and the absorbance was measured at 540 nm, using Enzyme-linked immunosorbent assay (ELISA) microtitre plate reader. Absorbance of solvent control containing the same amount of methanol and DPPH radical solution was measured as well. The experiment was performed in triplicates and the percentage radical scavenging activity and IC_{50} were calculated (23).

$$\% \text{ Scavenging activity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

ACUTE TOXICITY STUDY:

The acute toxicity study of the compounds was evaluated in the animals by up and down stair case method (24). Animals were divided into groups each consist of six animals. Drug suspension was prepared using 0.5 % w/v carboxymethyl cellulose (CMC). Test compounds were given orally as suspension at a single dose of 500, 1000 and 2000 mg/kg body weight. Control group was treated with 0.5 % CMC. Animals were continuously observed for the first 72 h and then for 7 day (d) for behavioral changes, toxicity and mortality.

***IN VIVO* ANTI-INFLAMMATORY ACTIVITY**

The anti-inflammatory potential of the compounds are usually assessed by carrageenan induced paw oedema method in rats (25). The animal care and handling were carried out in accordance to the guidelines issued by the Institutional Animal Ethics Committee, Manipal. The study was undertaken after obtaining approval of Institutional Animal Ethics Committee (F. No: IAEC/KMC/45/2009-2010). The acute toxicity study of the compounds was evaluated in the animals by up and down stair case method. The dose was selected based on the acute toxicity study reports. 1/10th of the safe dose was considered for this study. The animals were weighed, marked for identification and divided into groups, each containing 6 animals. The rats were starved for 18 h prior to the experiment. 0.5 % CMC was used to prepare the standard drug and test compounds suspension. Oedema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 mL of aqueous carrageenan into their foot pads. All the test compounds and the standard drug were administered orally, 1 h before the carrageenan injection. The paw volume of each rat was measured using a digital plethysmometer, just before the carrageenan injection (0 h) and then hourly for 5 h of post administration of the carrageenan. The increase in the paw volume at the 1st to 5th h were calculated and the results were expressed as mean increase in paw volume ± S.E.M. Results were analyzed by using one-way analysis of variance (ANOVA) followed by multiple comparison by post-hoc Dunnett's test.

Percentage Inhibition (%) = 100(1- (a-x/b-y))

Where, a = mean paw volume of treated animals after carrageenan injection

x = mean paw volume of treated animals before carrageenan injection

b = mean paw volume of control animals after carrageenan injection

y = mean paw volume of control animals before carrageenan injection

3. RESULTS:

Synthesis and characterization of fused 2,3-disubstituted benzopyrimidin-4-one derivatives

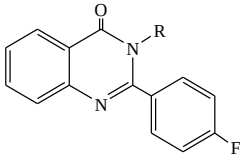
The synthesis of hitherto unreported title compounds were carried out, as outlined in Figure 1. The molecular weight, % yield, time for microwave irradiation (MWI) in min, melting point, R_f value and UV absorption (λ_{max}, nm) of all the synthesized compounds were shown in Tables 2-5.

Table 5. Physical characteristics of compounds BP 1-6

Compound Code	Molecular weight (Da)	Yield (%)	MWI Reaction time (min)	Melting point (ºC)	R_f	UV λ_{max} (nm)
BP-1	368	80	11	130	0.89	205
BP-2	348	86	10	178	0.78	206

BP-3	364	60	5	156	0.65	232
BP-4	350	87	10	145	0.81	205
BP-5	350	82	9	138	0.73	212
BP-6	311	78	6	92	0.86	206

General characterization of compounds 3-substituted-2-(4-fluorophenyl)benzopyrimidin-4-ones (BP 1-6)



A group of 3-substituted-2-(4-fluorophenyl)-benzopyrimidin-4-ones (BP 1-6) were synthesized, as outlined in Scheme 1 using *N*-(4-fluorophenyl)-anthranilic acid and different primary amines under microwave irradiation. Characterization of compounds was carried out by spectral analysis and the results are given in Figures 7-9. IR absorption of compounds BP 1-6 reported the presence of hetero aromatic -C═N- and C═O str. bands at 1438 - 1599 cm^{-1} and 1635 - 1670 cm^{-1}, respectively. Further, the presence of -CH_2- group was confirmed by the -CH- str. at 2924 cm^{-1}. Typically, the IR absorption spectrum of compound BP-1 displayed a strong peak at 1240 cm^{-1} owing to the presence of two fluoro substitutions in the molecule. In ^{1}H NMR spectrum of compound BP-6, the protons of the methyl groups were deshielded by the nitrogen and resulted for peak in the downfield, as shown in Figure 9b. An intense peak at δ 2.1 - 2.3 ppm represented the protons of the methyl groups. Further, the methylene protons resonated at δ 2.6-2.7 ppm and 3.0-3.2 ppm. The mass spectra of compound BP-2 exhibited the M^+ ion peak at *m/z* 348. Loss of Cl from -C_6H_4Cl fragment gave a peak at *m/z* 76, accounting for -C_6H_4 fragment (Figure 8b). The above data supported the proposed structures of compounds BP 1-6.

BIOLOGICAL ACTIVITIES:

Antioxidant activity of the synthesized fused benzopyrimidin-4-one derivatives

The DPPH• accepts electrons or hydrogen radicals to form a stable diamagnetic molecule exhibiting the colour change from purple to yellow and the results are expressed as the ability of the DPPH• to undergo reduction at 540 nm (26). In the present study, the test compounds showed moderate antioxidant activity. The percentage radical scavenging activity and the IC_{50} values were given in the Table and figure 2 respectively.

Figure 2. IC_{50} value of 2-(4-fluorophenyl)-3-substituted-benzopyrimidin-4-ones (BP 1-6)

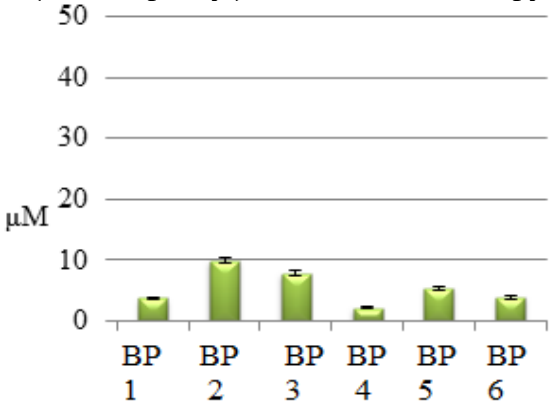


Table 6. Percentage antioxidant activity of 2-(4-fluorophenyl)-3-substituted benzopyrimidin-4-ones (BP 1-6)

Conc. (µg/mL)	Percentage antioxidant activity						
	BP-1	BP-2	BP-3	BP-4	BP-5	BP-6	Ascorbic acid
1000	40.38	17.33	20.66	56.85	30.66	41.52	90.31
500	26.80	8.83	12.10	43.51	23.05	21.99	89.15
250	25.30	5.78	8.530	36.20	19.05	12.13	89.70
125	21.95	5.20	7.06	32.00	16.00	7.06	89.69

62.5	17.37	5.14	6.06	29.59	12.40	5.05	89.59
31.25	12.46	3.32	4.43	24.83	7.94	2.49	65.92

ANTI-INFLAMMATORY ACTIVITY:

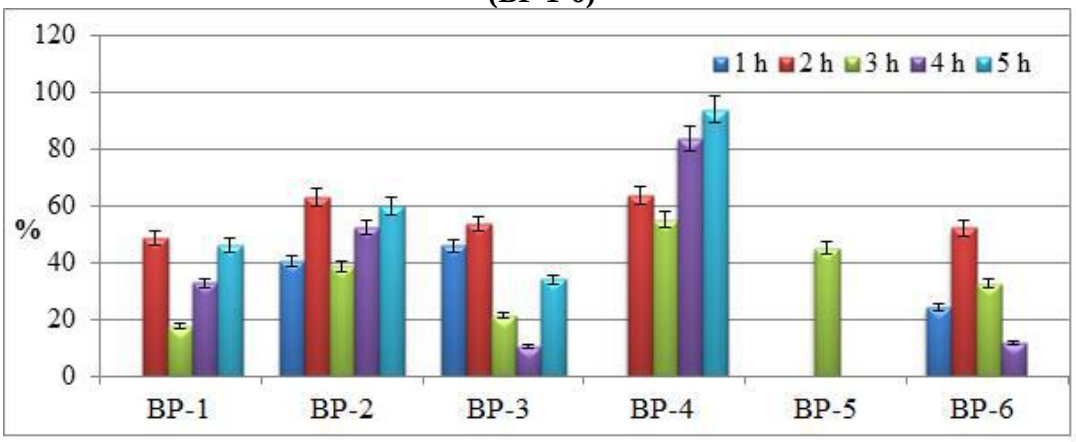
Inflammation is an immediate complex biological response of harmful stimuli. It is characterized by the accumulation of fluids and leukocytes which lead to oedema and pain (27). Tissue injury causes acute and chronic inflammation, which is mediated by the release of autocoids and varied mediators, such as, interleukins, interferon and tumor necrosis factor-α respectively (28). Non-steroidal anti-inflammatory drugs (NSAIDS) are commonly used for the treatment of pain and inflammation; however, on prolonged usage, NSAIDS may cause gastrointestinal ulceration and bleeding (29-30). Several drugs have been synthesized so far; however, they suffer from side effects. Therefore, it is important to find new anti-inflammatory drug with a potential for clinical use and not associated with adverse effects. Anti-inflammatory potential of 2-(4-fluorophenyl)-3-substituted benzopyrimidin-4-ones (BP 1-6) was studied by introducing six different groups at the 3rd position. The mean increase in the paw volume was measured and the percentage reduction of paw oedema of the test compounds was calculated. The results were given in the table and figure respectively.

Table 7. Effect of 2-(4-fluorophenyl)-3-substituted-benzopyrimidin-4-ones (BP 1-6) on mean increase in the paw volume

Code	Mean increase in the paw volume (mean ± S.E.M)				
	1 h	2 h	3 h	4 h	5 h
BP-1	0.10 ± 0.01	0.15 ± 0.00	0.16 ± 0.02	0.11 ± 0.02	0.06 ± 0.01*
BP-2	0.05±0.01***	0.11± 0.03	0.12 ± 0.01***	0.08 ± 0.01	0.05 ± 0.01**
BP-3	0.05±0.00***	0.14 ±0.00	0.15 ± 0.01*	0.15 ± 0.02	0.08 ± 0.01
BP-4	0.09 ± 0.01	0.11± 0.01	0.09 ± 0.01**	0.02 ± 0.03*	0.00 ± 0.01***
BP-5	0.12 ± 0.02	0.30± 0.02	0.11 ± 0.00*	0.16 ± 0.00	0.12 ± 0.01
BP-6	0.07 ± 0.00*	0.14 ±0.00	0.13 ± 0.01**	0.14 ± 0.00	0.17 ± 0.01
Control	0.09 ± 0.00	0.30 ±0.14	0.20 ± 0.03	0.16± 0.04	0.12 ± 0.03

NI: No increase in the paw volume; * p <0.05; ** p <0.01; *** p< 0.001

Figure 3. Percentage anti-inflammatory activity of 2-(4-fluorophenyl)-3-substituted-benzopyrimidin-4-ones (BP 1-6)



4. DISCUSSION:

Antioxidant activity of the compounds BP 1-6

The free radical scavenging ability of 2-(4-fluorophenyl)benzopyrimidin-4-ones containing different substitutions at the 3rd position such as, fluoro and chloro substituted phenyl or 2-(dimethylamino)ethyl moiety (BP 1-6) was

tested, the compound BP-4, bearing 4-chlorophenyl substitution showed a favourable antioxidant profile with the percentage radical scavenging of 56.85 at 1000 μg/mL as shown in Table 6. Further, the compound showed 50 % radical scavenging at 2.13 μM (Figure 2). Similarly, compounds containing 3-chloro-4-fluorophenyl (BP-1) and 2-(dimethylamino)ethyl (BP-6) substitutions showed antioxidant potential with 50 % radical scavenging at 3.7 and 3.8 μM, respectively. However, other compounds in this series did not show any promising radical scavenging activity. The above data revealed that, the antioxidant potential of benzopyrimidin-4-one system could be improved based on the presence of electron withdrawing substituents such as, 4-chlorophenyl, 3-chloro-4-fluorophenyl and *N*,*N*-dimethylaminoethyl functional groups at the 3rd position..

Anti-inflammatory activity

Among the tested compounds (BP 1-6), 3-(4-chlorophenyl)-2-(4-fluorophenyl) benzopyrimidin-4-one (BP-4) did not inhibit the paw oedema at the 1st h, as given in Table 7. However, it showed a reduction in the oedema at 55 - 94 % inhibition at 2nd to 5th h, as depicted in Figure 3. Further, the result was supported by its free radical scavenging ability, as shown in Table 7. However, compound BP-5, having 3-chlorophenyl substitution at the 3rd position showed activity at the 3rd h with reduction in the oedema at 45 % and other compounds in this series showed 10 - 64 % activity. The above results suggested that the increase in the anti-inflammatory potential of the benzopyrimidin-4-one could be because of the presence of the high electron withdrawing nature of 4-chlorophenyl and 4-fluorobenzyl substitutions at the 3rd position.

5. ACKNOWLEDGEMENTS:

The authors are thankful to AICTE, New Delhi for providing the research grant and also we express our gratitude to The Principal, RR College of Pharmacy and the management for providing the facilities to publish this work.

REFERENCES:

1. Wolfe J.F., Rathman T.L., Sleevi M.C., Campbell J.A., Greenwood T.D., (1990): Synthesis and anticonvulsant activity of some new 2-substituted 3-aryl-4(3H)-quinazolinones: Journal of medicinal chemistry, 33, 161-166.
2. Mosaad M.S., Mohsen K.M., Emad K.M., Abotaleb N., Salwa N.M., Marwa A.F., (2010). Novel 6,8-dibromo-4-benzopyrimidinone derivatives of promising anti-inflammatory and analgesic properties. Acta Pol Pharm, 67, 159–171.
3. Alagarsamy V., Raja Solomon V., Murugan M., Sankaranarayanan R., Periyasamy R., Deepa R., (2008) Synthesis of 3-(2-pyridyl)-2-substituted-benzopyrimidin-4-ones as new analgesic and anti-inflammatory agents. Biomedicine and Pharmacotherapy, 62, 454- 461.
4. Avinash P., Swasthika G., Sanjay S., (2010) Synthesis and antiulcer activity of 2-[5-substituted-1-*H*-benzo(*d*)imidazol-2-yl sulfinyl]methyl-3-substituted benzopyrimidine-4- ones. Journal of Chemical Science, 122(3), 443–450.
5. Dahiya R., Kumar A., Yadav R., (2008) Synthesis and biological activity of peptide derivatives of iodoquinazolinones/nitroimidazoles. Molecules, 24,13(4):958-76.
6. Thimmegowda N.R., Nanjunda Swamy S., Kumar C.S., Kumar Y.C., Chandrappa S., Yip G.W., (2008) Rangappa KS. Synthesis, characterization and evaluation of benzimidazole derivative and its precursors as inhibitors of MDA-MB-231 human breast cancer cell proliferation. Bioorg Med Chem Lett, 1;18(1), 432-5.
7. Chatrasal S.R., Sanjeev K., Ashok K., (2010) Synthesis and antifungal activity of newer substituted Benzopyrimidinones. Int J ChemTech Res, 2(3), 1653–1660.
8. Srivastava V.K., Satsangi R.K., Kumar P., Kishore K., (1980) Monoamine oxidase inhibitory activity of -2-aryl-(50-chlorobenzophenon-20-yl)benzopyrimidin-4-ones. Indian J Physiol Pharmacol, 24, 361–363.
9. Ponnilavarasan I., Swastika G., Vijay P., (2010) Design and synthesis of novel benzopyrimidinone derivatives as broad spectrum anticonvulsant and antimicrobial agent. J Pharm Res., 3, 703–706.
10. Azam M.A., Kumar B.R.R., Shalini S., Suresh B., Reddy T.K., Reddy C.D., (2008) Synthesis and biological screening of 5-{[(4,6-disubstituted pyrimidine-2-yl)thio]methyl}-*N*-phenyl-1,3,4-thiadiazol-2-amines. Indian Journal of Pharmaceutical Sciences, 70(5), 672–677.
11. Cao S.L., Feng Y.P., Jiang Y.Y., Liu S.Y., Ding G.Y., Li R.T., (2005) Synthesis and in vitro antitumor activity of 4-benzopyrimidinone derivatives with dithiocarbamate side chains. Bioorg Med Chem Lett., 15:1915–1917.
12. Siddiqui A.I.S.M., Macedo T.S., Moreira D.R.M., Leite A.C.L., Soares M.B.P., Azam A., (2014) Design, synthesis and biological evaluation of 3-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl]-propionic acid hydrazones as antiprotozoal agents. Eur J Med Chem., 75, 67–76.

13. Vachala S.D., Hurmath U., Essaki K., (2008) Synthesis and antibacterial activity of some 2,3-disubstituted benzopyrimidin-4 ones. Indian J Heterocycl Chem., 17, 347–350.
14. Yadagiri B., Holagunda U.D., Bantu R., Nagarapu L., Guguloth V., Polepally S., Jain N., (2014) Rational design, synthesis and anti-proliferative evaluation of novel benzosuberone tethered with hydrazide–hydrazones. Bioorg Med Chem Lett, 24, 5041–5044.
15. Griffin J., Srinivasan S., Bowman K., Kalvert H.A., Curtin N.J., Newel D.R., Pemberton L.C., Golding B.T., (1998) Synthesis and biological properties of benzopyrimidinone inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP). J Med Chem., 41, 5247–5256.
16. Banihashemi A., Pourabbas B., Karali N., Ilhan E., Gursoy A., Kiraz M., (1998) New cyclohexylidenehydrazide and 4-aza-1- thiaspiro[4.5] decan-3-one derivatives of 3-phenyl-4-benzopyrimidinones. II Farmaco., 53, 346–349.
17. McNamara D.J., Berman E.M., Fry D.W., Werbel L.M., (1990) Potent inhibition of thymidylate synthase by two series of non-classical benzopyrimidines. Journal of Medicinal Chemistry, 33, 2045-2051.
18. Varma R.S., Meshram H.M., (1997) Solid state cleavage of semicarbazones and phenylhydrazones with ammonium persulfate-clay using microwave or ultrasonic irradiation. Tetrahedron letters., 17, 38(46), 7973-6.
19. Liu G., Yang S., Song B., Xue W., Hu D., Jin L., Lu P., (2006) Microwave Assisted Synthesis of N-Arylheterocyclic Substituted-4-aminoquinazoline Derivatives. *Molecules.*, 11(4), 272-278.
20. Ramsbeck D., Buchholz M., Koch B., Böhme L., Hoffmann T., Demuth H.U., Heiser U., (2013) Structure-activity relationships of benzimidazole-based glutaminyl cyclase inhibitors featuring a heteroaryl scaffold. J Med Chem, 56(17), 6613-25.
21. Mahadik K.R., and Bhosale S.H. A hand book of practical chemistry. Nirali Prakashan, Pune. 2008 Nov 5: 120.
22. Mazaahir K., and Akkaldeomishra., (2004) An expeditious synthesis of 3,4-dihydrobenzo[2,3-*d*]pyrimidines using inorganic solid supports. Journal of Serbian Chemical Society. 69(4), 247-254.
23. Hemant R., Jadav, Bhutani K.K., (2002) Antioxidant properties of Indianmedicinal plants.Phytother Res, 16, 771-73.
24. Ghosh MN. *Fundamentals of Experimental Pharmacology*. 2d ed. Calcutta: Scientific Book Agency; 1984.
25. Winter C.A., Risley E.A., Nuss G.W., (1962) Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med., 111, 544-7.
26. Lee Y.R., Woo K.S., Kim K.J., Son J.R., Jeong H.S, (2007) Antioxidant Activities of Ethanol Extracts from Germinated Specialty Rough Rice. Food Sci Biotechnol. 16, 765-770.
27. Ferrero-Miliani L., Nielsen O.H., Andersen P.S., Girardin S.E., (2007) Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. Clin Exp Immunol., 147(2), 227-35.
28. Jacquelyn G., Black,, Laura J., Black., (2017) Microbiology: Principles and Explorations, 10: 446-467.
29. Allison M.C., Howatson A.G., Torrance C.J., Lee F.D., Russell R.I., (1992) Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs. N Engl J Med, 10, 327(11):749–754.
30. Wolfe M.M., (1998) Future trends in the development of safer nonsteroidal anti-inflammatory drugs. Am J Med, 105 (5A), 44S-52S.