Synthesis, characterization and biological evaluation of pyrazole containing benzophenones derivatives

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Abstract: **The synthesis of new pyrazole containing benzophenone derivatives was achieved by the Friedel-Craft acylation of regioisomeric aryl pyrazole framework 7 in the presence of Lewis acid ($AlCl_3$). The structures were analysed by IR, ^1H NMR, and mass spectroscopic data. Anticancer evaluation of the synthesized compounds against breast carcinoma reveals the significant anticancer potential of compounds 9e, 9c, 9i, and 9h.**

Keywords: Benzophenone, Pyrazole, Anticancer

I. INTRODUCTION

The benzophenone motif is one of the important structures in medicinal chemistry. These are commonly found in several natural products, which exhibit diverse biological activities such as anti-inflammatory, anticancer, antimicrobial, antifungal, anti-HIV, antiviral, etc (**Figure 1**).[1] The various marketed drugs such as ketoprofen (**1**), fenofibrate (**2**), tolcapone (**3**), etc. also contains benzophenone scaffolds (**Figure 1**). Thus, benzophenone scaffolds received considerable attention by the researchers working in the field of medicinal chemistry.

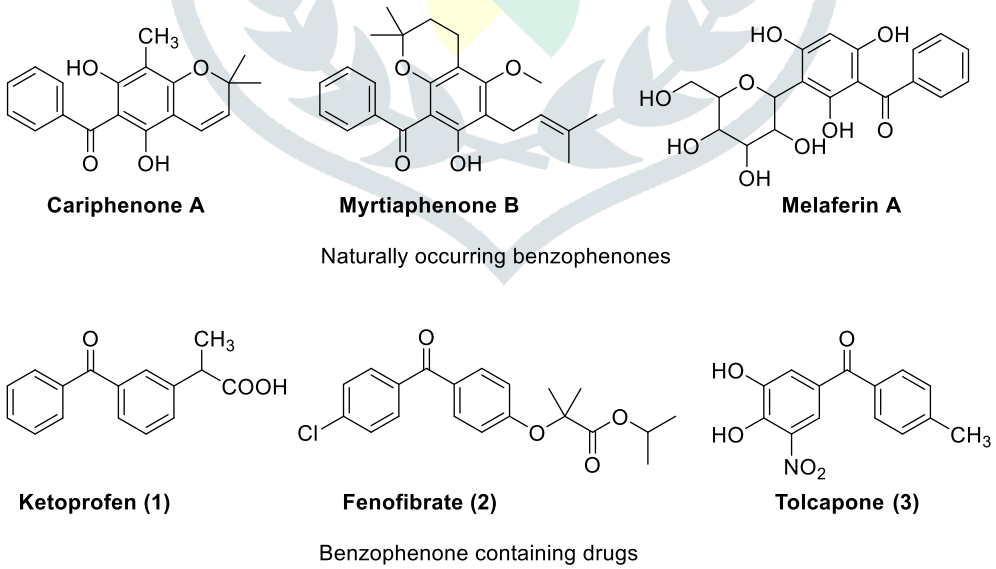


Figure 1. The naturally occurring and marketed drugs congaing benzophenone core

Pyrazoles are an important class of heterocyclic compounds for new drug development that also received substantial attention due to their wide spectrum of biological activities, such as anti-inflammatory, antifungal, anticancer, antiviral, etc.[2] Pyrazole derivatives also act as antiangiogenic, antidiabetic, anti-

hyperlipidemic, and anti-obesity agents.[2] Some drugs containing pyrazole heterocycles, such as ruxolitinib (**4**),[3] crizotinib (**5**),[4] celecoxib (**6**)[5] have created some hope for the cancer patients (**Figure 2**).

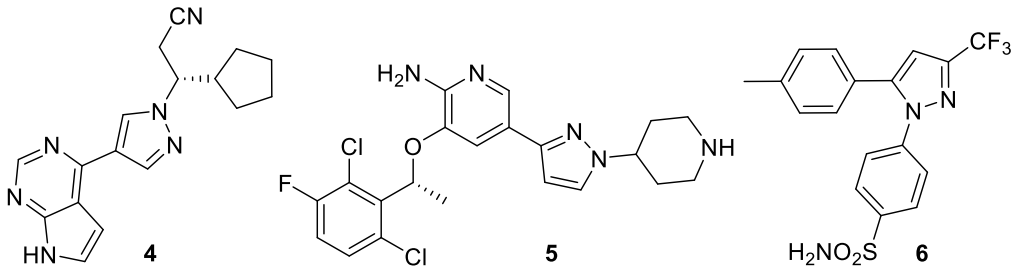


Figure 2. The representative pyrazole containing marketed drugs

Based on these interesting biological activity profiles of benzophenone and pyrazole derivatives, in the present work we made an attempt to synthesize a series of benzophenones derivatives containing pyrazole framework as a pendent group and evaluated as possible anticancer agents.

II. EXPERIMENTAL SECTION

Materials and Methods

All the chemicals used were of synthetic grade and procured from Sd-fine, Spectrochem and Aldrich chemicals. Completion of the reactions was monitored by thin layer chromatography (TLC) using E-Merck 0.25 mm silica gel plates. Visualization was accomplished with UV light and or iodine chamber. All the solvents were dried using appropriate drying agents before use. Melting points were determined by open end capillary method and are uncorrected. All the ^{1}H NMR spectra were recorded in DMSO-d_6 / $CDCl_3$ and chemical shifts in ppm were reported on instrument Bruker AV-400 MHz for ^{1}H NMR relative to TMS as an internal standard. The IR spectra were recorded on Shimadzu FT-IR spectrophotometer by using 1% potassium bromide discs. The electron ionization mass spectra were recorded on Agilent 1100 series.

SYNTHESIS

General procedure for the synthesis of benzophenone derivatives

A solution of 1-methyl-3-(2,4,6-trimethoxy-phenyl)-1*H*-pyrazole **7** (1.24 gm, 5 mmol) in dry dichloromethane (10 ml) was added to a cooled (5^{o}C) solution of anhydrous $AlCl_3$ (1.33 gm, 10 mmol) in dry dichloromethane (25 ml) under anhydrous condition. The resulting mixture was cooled to 0^{o}C, stirred vigorously, and a solution of desired benzoyl chloride (6 mmol) in dry dichloromethane (10 ml) was added slowly during 1 h. The stirring was continued for 2h at 0^{o}C and stirring continued at room temperature for 48 h. After completion the reaction (TLC), resulting mixture was poured over 50gm crushed ice and treated

with concentrated HCl (2.5 ml). The mixture was heated in water bath for 30 min, dichloromethane being allowed to distill away, cooled and the aqueous layer was extracted with diethyl ether (2x25 ml). The combined organic layer was washed with Na_2CO_3 solution (5%, 15 ml) and then extracted with NaOH (5%, 2x25 ml). The cooled clear alkaline solution was neutralized with dilute HCL and the separated solid was filtered, washed with water and recrystallized from methanol to obtain desired benzophenone derivative in pure form.

Spectral data of representative compounds:

(2-hydroxy-4,6-dimethoxy-3-(1-methyl-1H-pyrazol-3-yl)phenyl)(phenyl)methanone (**9a**): MP: 140-142 ^oC; IR (KBr): 3435, 2982, 2946, 1622, 1594, 1578, 1470, 1287, 1141 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.55-7.60 (m, 3H, 2xAr*H*, Pyr-*H*), 7.39-7.50 (m, 3H, 3xAr-*H*), 6.31 (s, 1H, Ar-*H*), 6.05 (s, 1H, Pyr-*H*), 3.86 (s, 3H, O*CH*$_3$), 3.71 (s, 3H, O*CH*$_3$), 3.59 (s, 3H, N*CH*$_3$); MS (ESI): m/e 339 (M+1).

Anti-cancer activity

Protocol for MTT assay [6] (%Inhibition): MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrzole) is the colorimetric assay for measuring the activity of enzymes that reduce MTT or close dye to formazan dye giving a purple color. A main application allows assessing the viability (cell counting) and proliferation of cells (cell culture assays). These reductions take place only when reductase enzymes are energetic and therefore conversion is often used as measure of viable (living) cells. It can be used to govern cytotoxicity of potential medicinal agents and toxic materials, since those agents would stimulate or prevent cell viability and growth.

The cell line was maintained in MEM medium supplemented with 10% fetal bovine serum. The cells were plated at a density of 1×10^5 cells per well in a 96-well plate, and cultured for 24 h at 37°C. The cells were afterward exposed to 50, 20, 10 µg/mL. The plates were incubated for 48 h, and cell proliferation was measured by adding 10 µL of MTT (thiazolyl blue tetrazolium bromide) dye (5 mg mL^{-1} in phosphate-buffered saline) per well. The plates were incubated for a further 4 h at 37°C in a humidified chamber containing 5% CO_2. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 200 µL DMSO, and absorbance was read at 490 nm. The results were compared with the standard drug inhibitors 5 flurouracil (20µg/mL). Lastly percent cytotoxicity of the compounds was calculated by using following formula.

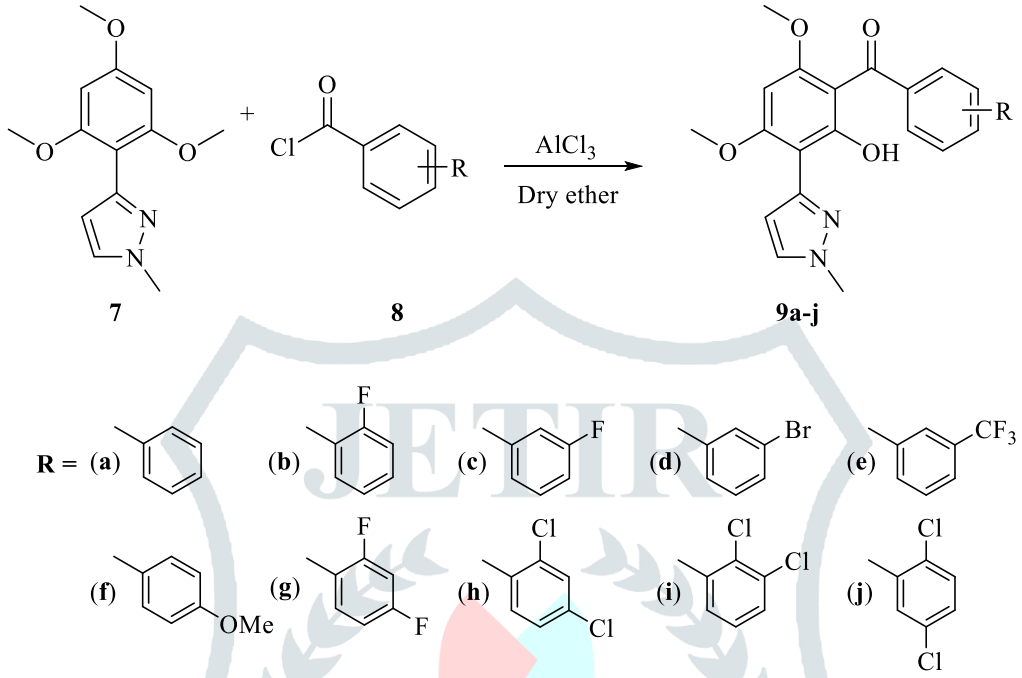
$$\textbf{Percent Cytotoxicity} = \frac{\text{Reading of control - Reading of treated cells}}{\text{Reading of control}} \times 100$$

III. RESULTS AND DISCUSSION

Chemistry

Synthesis of target molecules (**9a-j**) was achieved by the Friedel–Craft reaction of aryl pyrazole framework **7** with various acyl chlorides in the presence of anhydrous aluminium chloride under inert atmosphere

(**Scheme 1**). Regioselective synthesis of aryl pyrazole framework **7** was achieved as per our reported method[7] in the presence of sodium hydride and methyl iodide by modifying our previously reported method for the synthesis of regioisomer **6**.[8] All the synthesized compounds were characterized by IR, ^{1}H NMR and mass spectral data.



Scheme 1. Synthesis of benzophenone derivatives containing pyrazole pendent

Biological evaluation

All the synthesized compounds were screened for their anticancer potential against breast cancer cell line (MCF-7) by using MTT assay and the results are summarized in table 1. Among the compounds screened, compound **9e, 9c, 9i**, and **9h** possess good inhibitory potential against the MCF-7 cell line compared to the standard drug 5-fluoro uracil. All other compounds are moderate inhibitors. The SAR study reveals that, compounds bearing electron withdrawing groups such as trifluoromethyl, fluoro, chloro showed significant inhibition of MCF-7 cell line.

Table 1. Anticancer activity of the benzophenone derivatives against MCF-7

Compound	MCF-7 (% inhibition at 100μM)
9a	57
9b	60
9c	78
9d	65
9e	82
9f	55
9g	67
9h	71
9i	75
9j	63
5-FU	87

5-FU - 5-Fluorouracil, used as a positive control at 10 μM.

IV. CONCLUSION

In conclusion, a series of benzophenone derivatives (**9a-j**) bearing pyrazole pendent group have been synthesized from aryl pyrazole framework **7. The structures of a**ll the synthesized compounds were confirmed by IR, proton NMR and mass spectroscopic data. All the synthesized compounds were preliminary screened r anticancer potential against breast cancer cell line MCF-7. Among the compounds screened **9e, 9c, 9i**, and **9h** have shown significant inhibitory potential against the breast carcinoma.

REFERENCES

[1] U. M. Acuna, N. Jancovski and E. J. Kennelly, 2009. Polyisoprenylated benzophenones from Clusiaceae: potential drugs and lead compounds. Curr. Top. Med. Chem., 9:1560-1580; (b) S. B. Wu, C. Long and E. J. Kennelly, 2014. Structural diversity and bioactivities of natural benzophenones. Nat. Prod. Rep., 31:1158-1174.

[2] A. Ansari, A. Ali, M. Asif and Shamsuzzaman, 2017. Review: biologically active pyrazole derivatives. New J. Chem., 41:16-41

[3] J. Bronson, M. Dhar, W. Ewing, N. Lonberg, 2012. Chapter Thirty-One - To Market, To Market—2011. Annu. Rep. Med. Chem., 47:548.

[4] J. Bronson, M. Dhar, W. Ewing, N. Lonberg, 2012. Chapter Thirty-One - To Market, To Market—2011. Annu. Rep. Med. Chem., 47:525.

[5] (a) J. E. Kucab, C. Lee, C.S. Chen, C. Zhu, C.B. Gilks, M. Cheang, D. Huntsman, E. Yorida, J. Emerman, M. Pollak, S.E. Dunn, 2005. Celecoxib analogues disrupt Akt signaling, which is commonly activated in primary breast tumours. Breast Cancer Res. 2005, 7, 796-807; (b) A. T. Koki, J. L. Masferrer, 2002. Celecoxib: a specific COX-2 inhibitor with anticancer properties. Cancer Control, 9:28-35.

[6] J. McCauley, A. Zivanovic, D. Skropeta, 2013. Metabolomics Tools for Natural Product Discovery, 1055:191

[7] S. D. Ganapure, H. V. Chavan, R. M. Patil, 2018. Regioselective Synthesis and Structural Correlations of Aryl Pyrazole Framework, International Journal of Emerging Technologies and Innovative Research, 5(12): 706-711.

[8] B. P. Bandgar, H. V. Chavan, L. K. Adsul, V. N. Thakare, S. N. Shringare, R. Shaikh, R. N. Gacche, 2013. Design, synthesis, characterization and biological evaluation of novel pyrazole integrated benzophenones. Bioorg. Med. Chem. Lett. 23:912–916.