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Design, Synthesis, *In-silico* Drug Design of Novel Halo substitute Chromone Derivatives as Potent Hybrid Molecules for the Potential Treatment of Cancer.

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ABSTRACT

Chromones and benzopyrone derivatives are proved to have various pharmacological activities for their antitumor, antioxidant, and antimicrobial influence. Naturally occurring benzopyrones are subjected to a plenty of processing and structural modifications since they represent a promising field newer, safer, and effective agents. Halogenated chromones exert a marked action against cancers via different mechanisms. The length, size, and position of substitution of the side chain will control the final activity and potency of each derivative. The structures of the newly synthesized compounds were determined by HNMR, IR and mass spectrometry. The main activity was screened for colon cancer. The 6-chlorochromone-3-substituents showed higher effects when chlorophenyl bearing side chain is attached to the 6-chlorochromone nucleus or when a heterocyclic five-membered ring is employed.

Keywords: Colon cancer, Chromones, Heterocyclic, Benzopyrone.

INTRODUCTION

Cancer, or neoplasia, is a complex disease with multiple causes. Many intrinsic and extrinsic factors influence the development of cancer. Intrinsic or host factors include age, sex, genetic constitution, immune system function, metabolism, hormone levels, and nutritional status. Extrinsic factors include substances eaten, drunk, or smoked; workplace and environmental (air, water, and soil) exposures; natural and medical radiation exposure; sexual behaviour; and elements of lifestyle such as social and cultural environment, personal behaviour, and habits. Intrinsic and extrinsic factors can interact with one another to influence the development of cancer. In this article, we will discuss all the varied aspects of research that will ultimately lead to the prevention of cancer in man. [1].

Benzopyrone derivative (such as Coumarins, Chromones, Flavones) show a variety of biological activities. Based on the therapeutic activity, many structural modifications were carried out leading to semisynthetic compounds with better activity and lesser side effects. For example, Chrysin is a flavonoid natural product has an aromatase inhibitory activity [2]. While Chromones isolated from Eranthiscilicica (Ranunculaceae) possess antioxidant action. [3] Houghton noticed that the flavonoidal alkaloids and their derivatives, notably flavopiridol, have kinase-inhibition properties which could be applied to cancer chemotherapy. [4] A series of 3-methylchromones [chromone-3-(aryl substituted methyl)] was synthesized having different carbon side chain length (n) at 2-position (n = 0 to 3).

The compounds were tested using human hepatocellular liver carcinoma cell line (HepG2). Some analogues in the series proved to have protein tyrosine phosphatase inhibitory activity. [5] In addition, fluorinated Chromones were observed to have antiviral, anti-allergic, anti-inflammatory, and antifungals activities. [6] The antiproliferative activity of 4-methoxy-2-styrylchromone was observed as a microtubule-stabilizing antimitotic agent. It was tested using the human cell lines MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung cancer), the compound blocks the tumor cells in the G2/M phase of the cell cycle.[7] The compounds were tested using human hepatocellular liver carcinoma cell line (HepG2). Some analogues in the series proved to have protein tyrosine phosphatase inhibitory

activity. [8] In addition, fluorinated chromones were observed to have antiviral, antiallergic, anti-inflammatory, and antifungals activities. [9]

The hybrids were classified according to the position of the trimethoxyphenyl ring at C-2 or C-3 of the chromone and the presence or absence of a carbonyl as a linker between C-3 and the aryl ring. Many of those analogues showed a promising biologicalactivity. [10] The activity of 4-benzopyrone derivatives such as chromones and flavonoids against cancer cells could be through the following molecular mechanisms: A) Preventing Carcinogen Metabolic Activation, B) Antiproliferation, C) Cell Cycle Arrest [11] numerous anticancer drugs are currently marketed for treating different types of cancer.

However, owing to the increasing resistance, lack of target drug delivery, higher costs, and poor patient compliance, certain drugs are proving to be ineffective in curbing this gigantic disease. Our research project will shed some light on the newer possibilities of developing some alternative drugs for treating cancer. The proposed scheme may prove to be a guideline for synthesizing newer chromone derivatives as potential anticancer agents.

MATERIALS AND METHODS

Step 1: 5 Choro – 2 – Hydroxy aceto phenone was treated with excess sodium acetate placed in a 250 ml round bottom flask, then acetic anhydride was added in excess and the mixture was shaken uniformly. Refluxing of the mixture was carried out at 190 – 200^0 c .During boiling the contents were melting and dissolving slowly showing thick viscous foam that disappeared gradually with time producing a clear solution of deep orange- brown colour, the reaction was continued for 8-10 hours. The reaction mixture was then poured into crushed ice water with continuous stirring and kept in the fridge overnight. The solid product was filtered and washed thoroughly with distilled water, with diluted sodium carbonate solution and then with distilled water so that no more acetic smell was there. It was crystallized from methanol as yellow to pale orange crystals and tested by TLC.

Step 2: A quantity of step (1) compound was dissolved in 30 ml of absolute alcohol together with Benzaldehyde or equimolar quantity of different benzaldehyde derivatives. The mixture was stirred on a magnetic stirrer till complete dissolution. Alcoholic solution of potassium hydroxide was added to the mixture and stirring continued at room temperature for 48hrs. The mixture was poured into ice water neutralized by Hcl and the precipitate was filtered, washed and dried [12, 13, 14].

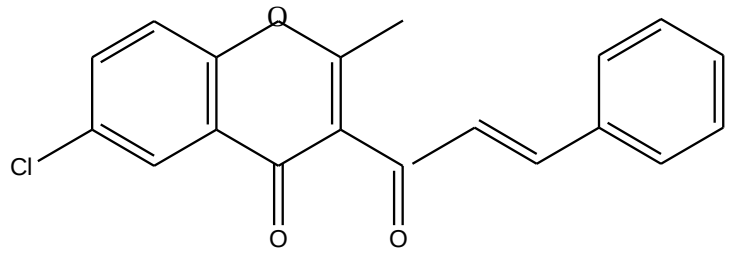
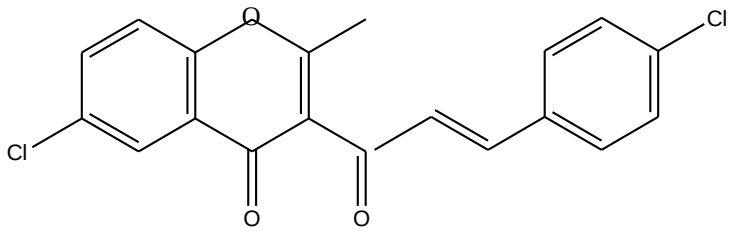
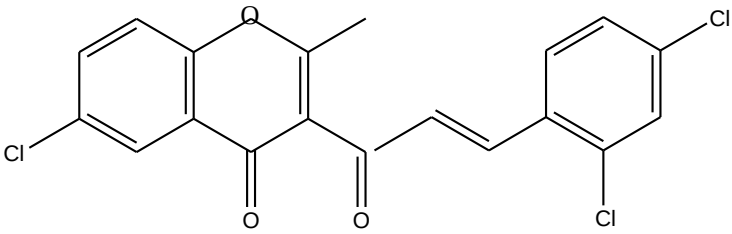
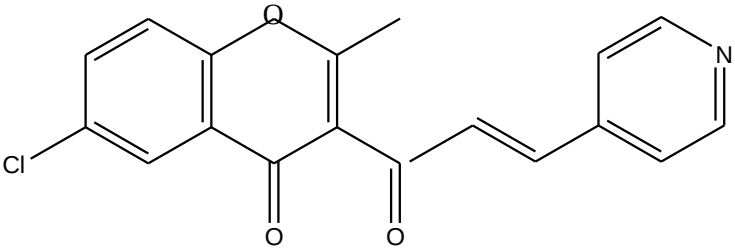
MTT cytotoxicity Assay: MTT is taken up by the viable cells and reduced to Formazan by succinate terazoliumreductase system that belongs to the mitochondrial respiratory chain functioning in metabolically active cells. Formazan formed ,is purple coloured water insoluble product that is largely impermeable to cell membranes , thus resulting in its accumulation within the healthy cells which is solubilized by adding Dimethyl sulphoxide (DMSO).The optical density (OD) of purple coloured solution developed was read using a conventional ELISA plate reader at 590nm(maximum absorbance). The ability of the cells to reduce MTT provides an indication of mitochondrial integrity and activity, which, in turn, may be interpreted as measure of viability and or cell number.

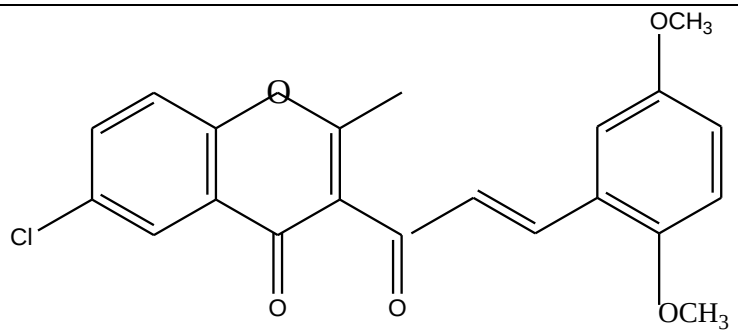
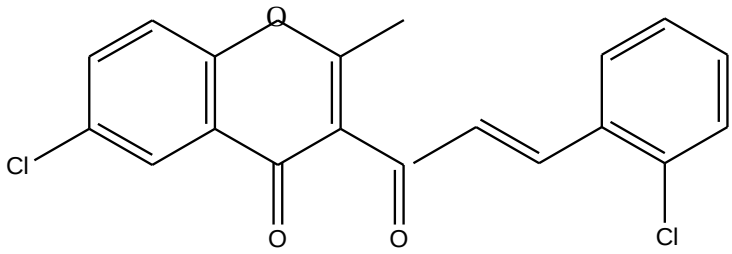
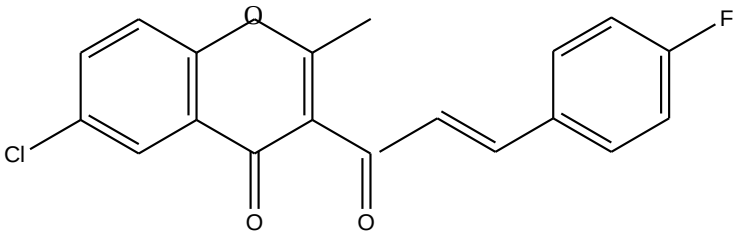
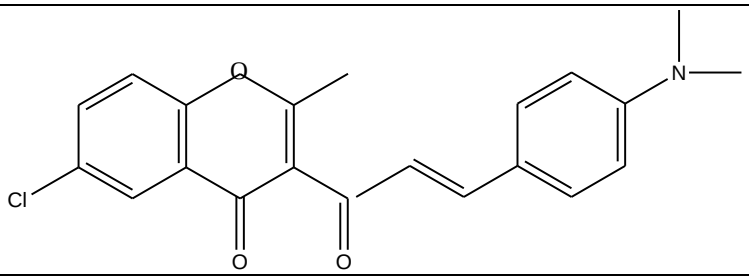
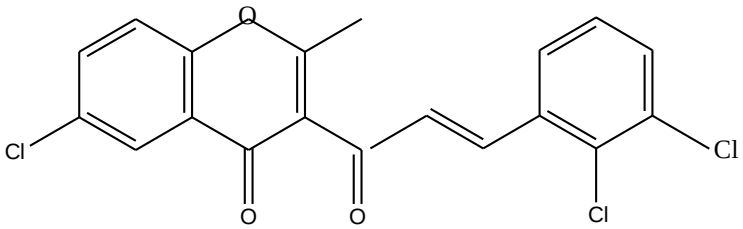
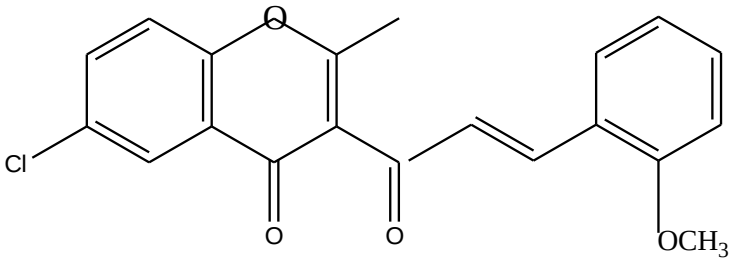
Procedure: Exponentially growing HCT-116 cells were harvested from T-25 tissue culture flask and a stock cell suspension was prepared. A sterile 96 well flat bottom tissue culture plate was seeded with 5×10^3 cells in 0.1 ml of MEM medium supplement with 10% FBS and allowed to attach for 24hrs. Cells were treated with different concentrations of test compound (50 – 400micro M) in triplicates and incubated for 48 hrs. The control group cells were treated with only the medium containing O.1%. (DMSO).The 30 microlitre of MTT reagent

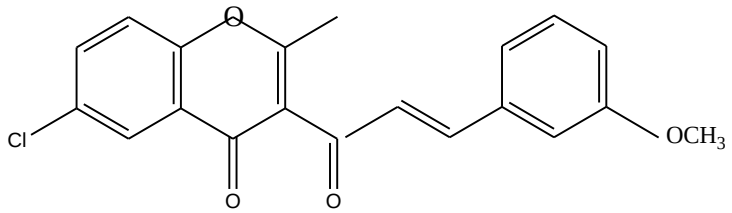
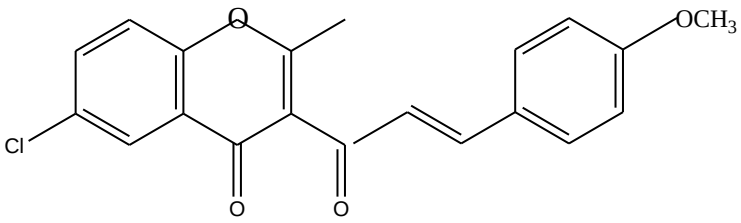
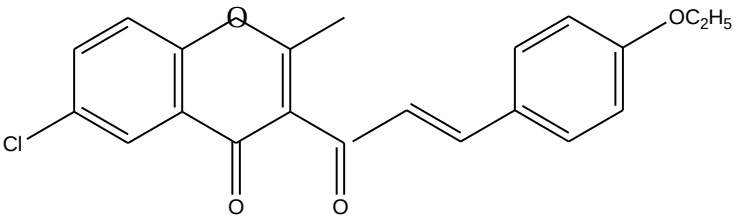
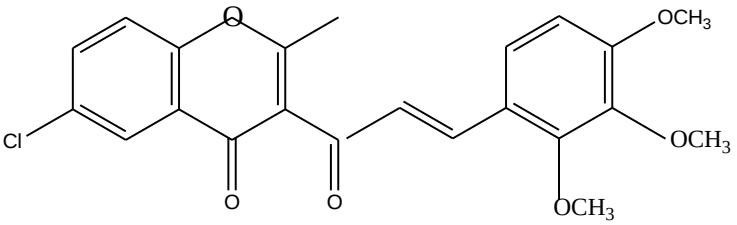
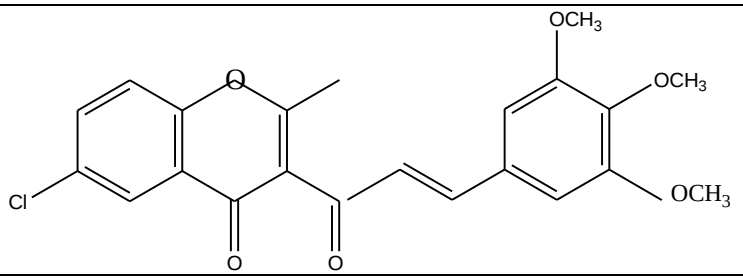
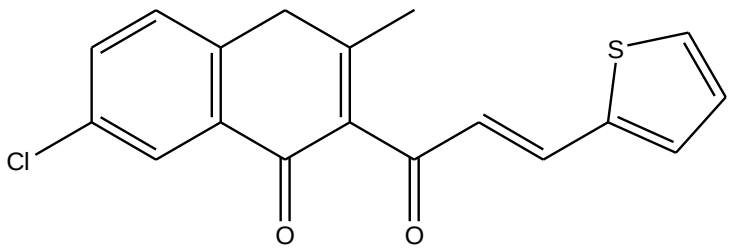
(4mg/ml) was added in to Wells and incubated for 4 hours at 37^0 c. After three hours of incubation, medium containing MTT was removed draining on tissue paper and the Formazan crystals formed in each well were dissolved in 100 microlitre of DMSO.The absorbance was measured by ELISA plate reader at 540 nm. Support by necessary tables, charts, diagrams and photographs. [15]

RESULTS

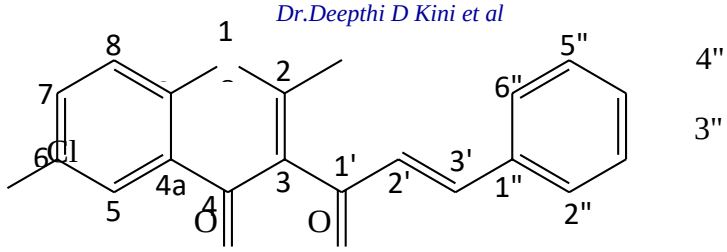
A number of acryloylchlorochromone analogues were synthesized via a two-step procedure using 5-chloro-2-hydroxyacetophenone as a starting material. Structural characterization was done using HNMR, Mass spectrometry and IR spectroscopy. The newly synthesized compounds were tested for anti-proliferative action on human colon cancer HCT-16 cells.

Sl.No	Structure	Name	MP
1.		6-chloro-2-methyl-3-(3-phenylacryloyl)-chromen-4-one	70 %
2.		6-chloro-2-methyl-3-[3-(4-chlorophenyl)-acryloyl]-chromen-4-one	65 %
3.		6-chloro-2-methyl-3-[3-(2,4-dichlorophenyl)-acryloyl]-chromen-4-one	58 %
4.		6- Chloro -2 methyl-3-[3-4-pyridyl) acryloyl] chromen-4-one.	72 %

5.		6-chloro-2-methyl-3-[3-(2,5-dimethoxyphenyl) -acryloyl]-chromen-4-one	75 %
6.		6-chloro-2-methyl-3-[3-(2-chlorophenyl)-acryloyl]-chromen-4-one	68 %
7.		6-chloro-2-methyl-3-[3-(4-fluorophenyl)-acryloyl]-chromen-4-one	62 %
8.		6-chloro-2-methyl-3-[3-(4-dimethylaminophenyl)-acryloyl]-chromen-4-one	51 %
9.		6-chloro-2-methyl-3-[3-(2,3-dichlorophenyl)-acryloyl]-chromen-4-one	48 %
10.		6-chloro-2-methyl-3-[3-(2-methoxyphenyl)-acryloyl]-chromen-4-one	73 %

11.		6-chloro-2-methyl-3-[3-(3-methoxyphenyl)-acryloyl]-chromen-4-one	70 %
12.		6-chloro-2-methyl-3-[3-(4-methoxyphenyl)-acryloyl]-chromen-4-one	77 %
13.		6-chloro-2-methyl-3-[3-(4-ethoxyphenyl)-acryloyl]-chromen-4-one	77 %
14.		6-chloro-2-methyl-3-[3-(2,3,4-trimethoxyphenyl)-acryloyl]-chromen-4-one	71 %
15.		6-chloro-2-methyl-3-[3-(3,4,5-trimethoxyphenyl)-acryloyl]-chromen-4-one	70 %
16.		6-chloro-2-methyl-3-[3-(2-thienyl)-acryloyl]-chromen-4-one	79 %

SPECTRALDATAINTERPRETATION
(For biologically active derivatives)



The parent structure

Compound ID	Concentration(µM)	**IC_{50}(µM)**
CLRM1	50	25.60
CLRM2	50	36.90
CLRM3	50	73.17
CLRM4	50	299.40
CLRM5	50	>400
CLRM6	50	23.98
CLRM7	50	101.60
CLRM8	50	>400
CLRM9	50	3.92
CLRM10	50	61.95
CLRM11	50	69.47
CLRM12	50	178.00
CLRM13	50	123.50
CLRM14	50	22.01
CLRM15	50	39.23
CLRM16	50	1.06
Doxorubicin	0.005	0.42

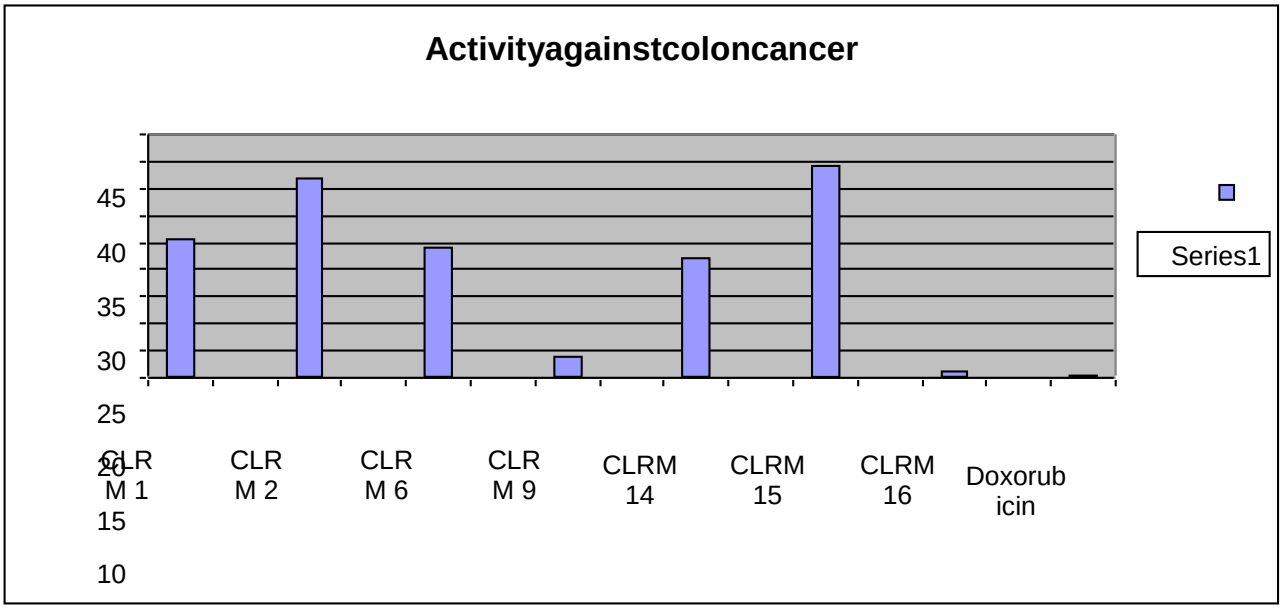
Biological Studies Antiproliferative Activity On Human Colon Cancer Cell Line Hct-

Contributions made towards increasing the state of knowledge in the subject newly synthesized compounds exhibited significant Pharmacological activity. The newly synthesized compounds were screened in HCT-116. And they were showing anticancer activity in different degrees. In HCT-116, seven derivatives were active (IC50 below 50 μM); namely, CLRM1, CLRM2, CLRM6, CLRM9, CLRM14, CLRM15, CLRM16. CLRM16 was of close IC50 to doxorubicin, and CLRM9 was the next.

Derivatives carrying basic substituents were the least active. For example, pyridine and dimethyl amino phenyl substituted acryloyl side chain gave very low activities.

It appeared that mono-methoxy derivative could show better activity than the dimethoxy substituent, especially if the methoxy groups are notadjacenttoeachothersasin2, 5-dimethoxyphenyl-acryloyl (CLRM5).

Increasing the length of the alkoxy group did not give a big change in the biological activity. For example, 4-ethoxyphenyl substituent showed a minor increase in activityincomparisonto4-methoxyphenylanalogue (CLRM12 andCLRM13respectively).



Graphical representation of IC50 values of CLRM derivatives on colon cancer cell line HCT-116.

DISCUSSION

In HCT-116, seven derivatives were active (IC50 below 50 μM); namely, CLRM1, CLRM2, CLRM6, CLRM9, CLRM14, CLRM15, CLRM16. CLRM16 was of close IC50 to doxorubicin, and CLRM9 was the next. Derivatives carrying basic substituents were the least active. For example, pyridine and dimethyl aminophenyl substituted acryloyl side chain gave very low activities. It appeared that mono-methoxy derivative could show

Better activity than the dimethoxy substituent, especially if the methoxy groups are not adjacent to each other's as in 2, 5-dimethoxyphenyl-acryloyl (CLRM5). Increasing the length of the alkoxy group did not give a big change in the biological activity. For example, 4-ethoxyphenyl substituent showed a minor increase in activity in comparison to 4-methoxyphenyl

analogue (CLRM12 and CLRM13 respectively).

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