Faculty of Science
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SYNTHESIS OF SOME NEW COUMARIN DERIVATIVES AND STUDY THEIR ANTITUMOR ACTIVITY

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For the Degree of
Doctor of Philosophy [Ph.D]
(In Chemistry)

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2018

Approval Sheet

Synthesis of some new coumarin derivatives and study their antitumor activity

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ACKNOWLEDGEMENT

First of all, I would like to extend due praise and thanks to ALLAH that this work has been completed.

I would like to express my sincere gratitude to ***Prof.Dr.*** **Hassan M. F. Madkour,** Professor of Chemistry Department, *Faculty of Science, Ain Shams University* for his continuous supervision, encouragement and kind support during the course of the present investigation.

My endless appreciation to ***Prof. Dr. Omaima Mohamed Abd Elhafez*****,** Professor of Natural Products Chemistry, Chemistry of Natural Compounds Department, National Research Centre for suggesting the point of research, constructive supervision, and her great support, faithful advice and unlimited efforts in this research.

I am also deeply grateful to **Prof. Dr. Ezz El-Din A. M. El-Khrisy** Professor of Natural Products Chemistry, Chemistry of Natural Compounds Department, National Research Cent**r**e for his kind supervision, valuable advices and encouragement through the work.

I wish to express my great thanks and appreciation to **Dr. Radwan S. El-Haggar,** Assistant professor Pharmaceutical Chemistry Department, Faculty of Pharmacy, Helwan University, for his fruitful contribution in this work. **Dr. Radwan** designed, carried out and interpreted the outcomes data of the biological evaluations.

Sincere thanks to **Dr. Wagdy M. Eldehna**, Lecturer of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University for carrying out Quantitative Structure activity relationship (QSAR).

I would like to express my thanks and appreciation to **prof.** ***Dr.*** **Laila. A. Rashed**, Professor of biochemistry and molecular biology, Department of

Biochemistry, Faculty of Medicine, Cairo University, for carrying out the *In vitro* cancer cell lines Studies

Words couldn't help me in expressing my deep appreciation to ***Professor Harri Lönnberg and Dr. Mikko Ora*** at University of Turku and Åbo Akademi University, Finland, for hosting me 8 months at his group and and kind help me to complete synthesis part from my thesis. Also, I am grateful to Prof. Dr. ***Daniel Abankwa***. Professor of Åbo Akademi university, Finland for carrying biology of K-ras.

I am thankful to all my colleagues at Chemistry of Natural Compunds Department- National Research Centre for emotional support and encouragement during completion of this work.

I would like to give my special thanks to **my wife**, **my mother** and **my brother** for the patience and moral support they provided throughout my research work.

Sincerely
Ahmed Sabt

Contents

List of Abbreviations

Arg	Arginine (R)
Arl2	The ADP-ribosylation factor-like 2(release factor)
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
DMTrCl	4,4- Dimethoxytrityl chloride
EtOAc	Ethylacetate
FLIM	Fluorescence lifetime microscopy
FRET	Forster resonance energy transfer
FTase	Farnesyl transferase enzyme
FTI	Farnesyl transferase inhibitors
GAPs	GTPase activating proteins
GEFs	Guanine nucleotide exchange factors
Glu	Glutamate (E)
HEK	Human Embryonic Kidney
HRMS	High resolution mass
HVR	C-terminal hypervariable region
IC_{50}	The half maximal inhibitory concentration
k-ras	Kirsten rat sarcoma viral oncogene
MAPK	Mitogen-activated protein kinase
Met	Methionine (M)
PDB	Protein Data Bank
PDEδ	Delta subunit of the phosphodiesterase 6
PI3K	phosphatidylinositol 3-kinase
QSAR	Quantitative structure activity relationship
Ras	Rat sarcoma (protein)
RMSD	Root mean standard deviation
SRB	Sulforhodamine B
siPDEδ	Specific inhibitors Delta subunit of the phosphodiesterase 6
TEA	Triethylamine
THF	Tetrahydrofurane
Tyr	Tyrosine (Y)

Abstract

Name: Ahmed Sabt Ibrahim Mohamed

Title: Synthesis of some new coumarin derivatives and study their antitumor activity

Coumarin was subjected to chlorosulfonation *via* chlorosulfonic aicd to yield coumarin-6-sulfonylchloride **1** which was utilized as a key intermediate to prepare new series from coumarin-6-sulfonamides derivatives. Therefore, compound **1** was refluxed in ethanol with sulfanilamide, pyridine sulfanilamide and/or 4-aminoacetophenone to afford the corresponding coumarin-6-sulfonamides **2 - 4**, repectively. N-(4-acetylphenyl)-2-oxo-2*H*-chromene-6-sulfonamide **4** reacted with phenyhydrazine derivatives in absolute ethanol and triethylamineto afford **5a-c**. On the other hand, when compound **4** reacted with *p*-toluene sulfonylhydrazide, compound **6** was isolated. When compound **4** was refluxed with thiosemicarbazide in the presence of ethanol and acetic aicd, the thiosemacarbaone **7** was obtained.

The reaction of thiosemacarbazone **7** with chloroacetone, ethyl-2-chloroacetoacetate, hydrozonylchlorides-p-Cl and p-CH_3, phencylbromide and/or 3-(2-bromoacetyl)-2*H*-chromen-2-one under reflux condition in dioxane and triethylamine gave the corresponding coumarinthiazole derivative **8-12**, repectively. Also, thiazolidinone derivatives were obtained *via* reaction of thiosemicarbazone **7** with bromoacetic acid and/or 2-bromopropanoic to afford the thiazolidinone derivatives **13** and **14**, repectively**.**

2-((6-hydroxyhexyl)oxy)isoindoline-1,3-dione **15** reacted with 4,4-dimethoxy tritryl chloride (DMTrCl) in dry pyridine to give **16**.

Compound **16** strirred with hydrazine hydrate at room temperature to give *O*-6-dimethoxytritylatedhexylhydroxylamine **17.** 3-acetylcoumarin condensed with compound **17** in the prescence of ethanol and pyridine to yield coumarin *O*-alkyl oximetritryl **18** and a subsequent conversion to **19** *via* the acid catalyzed detritylation process. Phosphitylation of **19** *via* three steps to give final product **20.** by similar way compound 4 reacted with aminooxy and then Phosphitylation to afford compound **23**.

Moreover , 6-bromohexanol **24** reacted with tritrylchloride in pyridine yielded 6-bromotritryl **25**. The later was subjected to react with 4-hydroxycoumarin to afford compound **26**. Detritrylation of compound **26** and then Phosphitylation to convert to the final target **28**.

Coumarin-3-carboxylate **29** was allowed to reflux with 6-aminohexanol in the prescence of ethanol and piperdine as a catalyst and afforded coumarin-3-carboxamide **30**. Compound **30** subjected to Phosphitylation process to yield final target **31.**

The newly synthesized compounds **2-14** were evaluated for their anti-proliferative activity against three human tumor cell lines HepG2, CaCo and MCF-7. The most active compounds **11** and **13** were further studied for their apotosis inducing properties. a 2D-*QSAR* model was performed on these synthesized compounds. also, the four final compounds from **20**, **23**, **28** and **31** were evaluated as potent KRAS-PDEδ interaction inhibitors. Computational docking of the compounds into the PDEδ crystal structure was performed.

Key words: Synthesis, Coumarin derivatives, Phosphitylation, apopotosis, anti-proliferative activity and k-ras.

SUMMARY

The present research work aimed to synthesize new series of coumarin-6-sulfonamides bearing Schiff's bases, thiazole or thiazolidinone moieties. In addition the coumarins derivatives linked to the protected phosphate moiety *via* a hexyl hydrocarbon chain were prepared. All the target compounds were subjected to antitumor activity assessement. Moreover, molecular docking and quantative structure-activity relationship were perforemed to help the experts to better predict the possible activity of target compounds.

Coumarin was subjected to chlorosulfonation by chlorosulfonic acid yielded coumarin-6-sulfonylchloride **1** which was utilized as a key intermediate to prepare new series from coumarin-6-sulfonamides derivatives. Therefore, compound **1** was refluxed in ethanol in presence of glacial acetic acid with sulfanilamide and/or pyridine sulfanilamide to afford the corresponding coumarin-6-sulfonamides **2** and **3**, repectively. Coumarin-6-sulfonylchloride **1** was allowed to react with 4-aminoacetophenone in presence of dichlormethane and pyridine to yield N-(4-acetylphenyl)-2-oxo-2*H*-chromene-6-sulfonamide **4 [cf.scheme1]**.

Moreover, Schiff's bases derivatives were prepared *via* condenation of N-(4-acetylphenyl)-2-oxo-2*H*-chromene-6-sulfonamide **4** with phenyhydrazine derivatives in absolute ethanol and triethylamine to afford **5a-c**. On the other hand, when compound **4** reacted with *p*-toluene sulfonylhydrazide, compound **6** was isolated. When compound **4** was refluxed with thiosemicarbazidein the presence of ethanol and acetic aicd,

the thiosemacarbazone **7** was obtained **[cf.scheme2]**.

The reaction of thiosemacarbazone **7** with chloroacetone under reflux condition in dioxane and triethylamine gave the methylthiazole derivative **8**. Also, when compound **7** was allowed to react with ethyl-2-chloroacetoacetate, the thiazolecarboxylate compound **9** was obtained. refluxing thiosemicarbazones **7** with different hydrozonylchlorides-p-Cl and p-CH_3, it gave coumarinthiazole derivatives **10a** and **10b[cf.scheme3].**

When thiosemicarbazone **7** was allowed to react with phencylbromide in the prescence of dioxane and triethylamine, compound **11** was isolated. Compound **7** reacted with 3-(2-bromoacetyl)-2*H*-chromen-2-one to give the thiazole derivative **12 [cf.scheme4].** Also, thiazolidinone derivatives were obtained *via* reaction of thiosemicarbazone**7** with bromoacetic acid and/or 2-bromopropanoic acid under reflux in acetic acid in the presence of an excess anhydrous NaOAc to afford the thiazolidinone derivatives **13** and **14**, repectively **[cf.scheme 4].**

Moreover, The newly synthesized compounds **2-14** were evaluated for theier anti-proliferative activity against three human tumor cell lines HepG2 hepatocellular carcinoma,CaCo cancer and MCF-7 breast cancer. In particular, HepG2 cancer cell line was more susceptible to the synthesized derivatives. Compounds **11** and **13** (IC_{50} = 3.48 ± 0.28 and5.03 ± 0.39μM, respectively) were found to be the most potent derivatives against HepG2 being more active than doxorubicin (IC_{50} = 5.43 ± 0.24). In addition, compound **14** showed the most promising results against MCF-7 with IC_{50} =10.62 ± 1.35μM. Moreover, cytotoxicity evaluation of the newly synthesized compounds in CaCo

revealed that compound **5a** exhibited significant antiproliferative activity with IC_{50} value 8.53 ± 0.72μM. Therefore, a 2D-*QSAR* model was performed on these synthesized compounds, to extract out the key regulatory features controlling the anticancer activity. The most active compounds **11** and **13** were further studied for their apoptosis inducing properties.

In the continuation of work, 2-((6-hydroxyhexyl)oxy)isoindoline-1,3-dione **15** reacted with 4,4-dimethoxytrityl chloride (DMTrCl) in dry pyridine and afforded 2-((6-(bis(4-methoxy phenyl) (phenyl) methoxy) hexyl)oxy)isoindoline-1,3-dione **16**. Compound **16** strirred with hydrazine hydrate at room temperature to give *O*-6-dimethoxytritylated hexylhydroxylamine **17** **[cf.scheme5]**.

On the other hand, 3-acetylcoumarin condensed with compound **17** in the prescence of ethanol and pyridine to yield coumarin *O*-alkyl oximetritryl **18** and a subsequent conversion to 3-(1-(((6-hydroxyhexyl) oxy) imino) ethyl)-2*H*-chromen-2-one **19** *via* the acid catalyzed detritylation process **[cf.scheme5]**.

Phosphitylation of **19** with 1-chloro-*N*,*N*-diisopropyl-1-methoxy-phosphinamine followed by tetrazole promoted displacement of the diisopropylamino ligand by 4-acylthio-2-hydroxymethyl-2-methyl-3-oxobutanoate and oxidation of the resulting phosphite triester to phosphate triester with iodine in THF/H_2O/2,6-lutidine completed the synthesis to give final product **20 [cf.scheme5].**

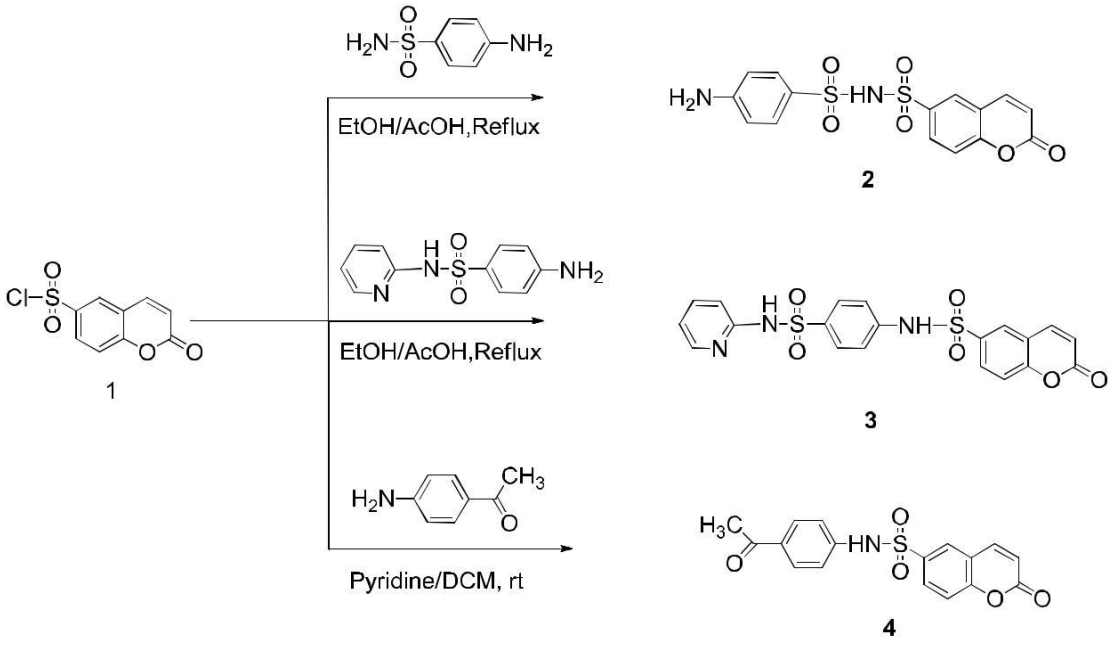
When compound **4** reacted with *O*-6-dimethoxytritylated hexylhydroxylamine **17,** *O*-alkyl oxime **21** was isolated. Compound **21** was stirred in dichloroacetic acid in prescene of dichlormethane and afforded hydroxyhexylcoumarin **22**. Compound **22** was subjected to the phosphitylation process by using three steps to yield the target coumarinhexylphosphate **23[cf.scheme6]**.

On the other hand, 6-bromohexanol **24** reacted with tritylchloride in pyridine yielded 6-bromotritryl **25**. The later was subjected to react with 4-hydroxycoumarin to afford compound **26**. Detritylation of compound **26** in the prescence of dichloroacetic acid and dichloromethane gave coumarin-4-hydroxyhexyl **27**. Similarly, compound **27** was converted to the final target **28** *via* Phosphitylation **[cf.scheme7]**.

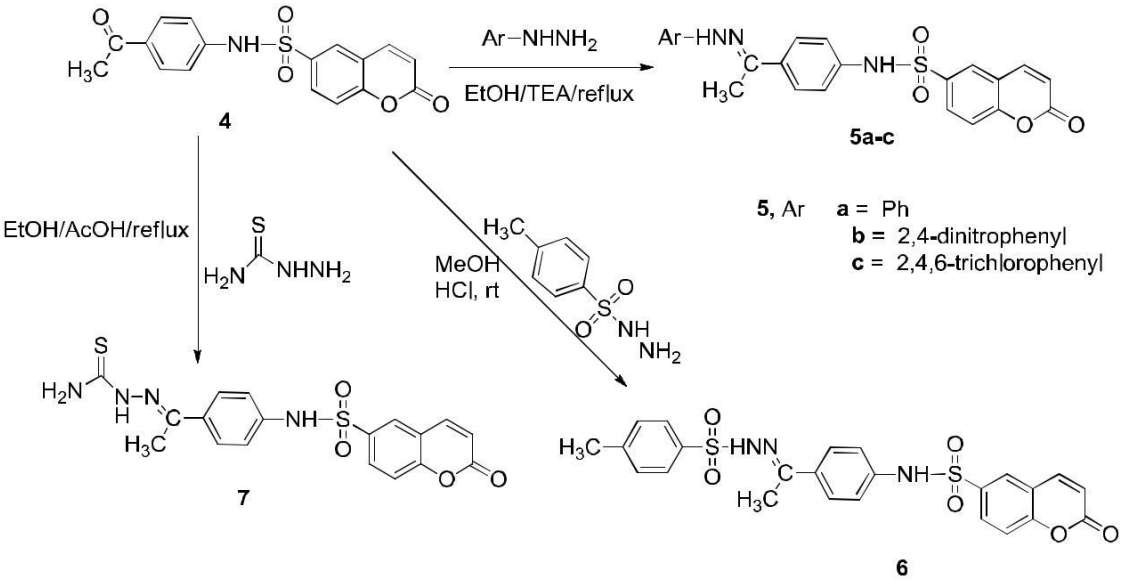
Coumarin-3-carboxylate **29** was allowed to reflux with 6-aminohexanol in the prescence of ethanol and piperdine as a catalyst and afforded coumarin-3-carboxamide **30**. Compound **30** reacted with 1-chloro-*N*,*N*-diisopropyl-1-methoxy phosphine amine followed by tetrazole promoted displacement of the diisopropylamino ligand by 4-acylthio-2-hydroxymethyl-2-methyl-3-oxobutanoate and oxidation of resulting phosphite triester to phosphate triester with iodine in THF/H_2O/2,6-lutidine completed the synthesis to give final target **31 [cf.scheme8]**.

In order to demonstrate the validity of these reported research information, the synthesized target compounds were screened for their antitumor activity. Therefore, the four final compounds of coumarin linked to the protected phosphate moiety *via* a hexyl hydrocarbon chain (**20**, **23**, **28** and **31**) were evaluated as potent KRAS-PDEδ interaction inhibitors. Computational docking of the compounds into the PDEδ

crystal structure was performed. Our model compound, Deltaflexin1, selectively disrupts K-Ras, but not H-Ras membrane organization. This selectivity profile is reflected in its anti-proliferative activity on breast and colorectal cancer cells.



Scheme 1



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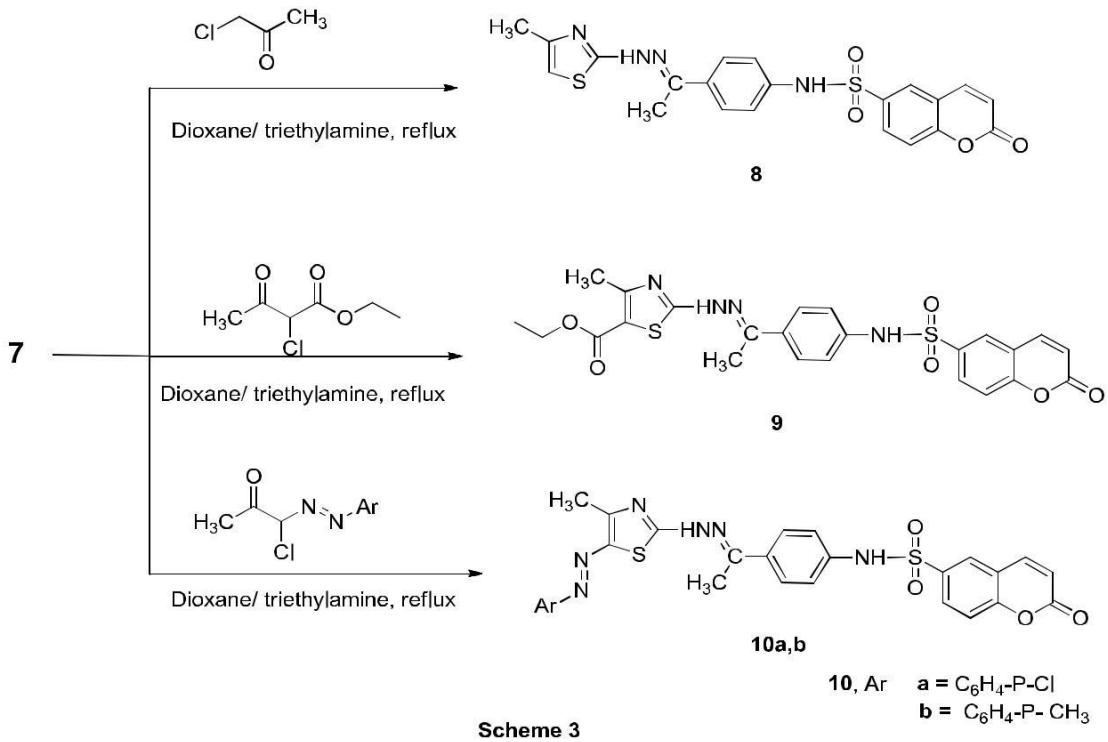

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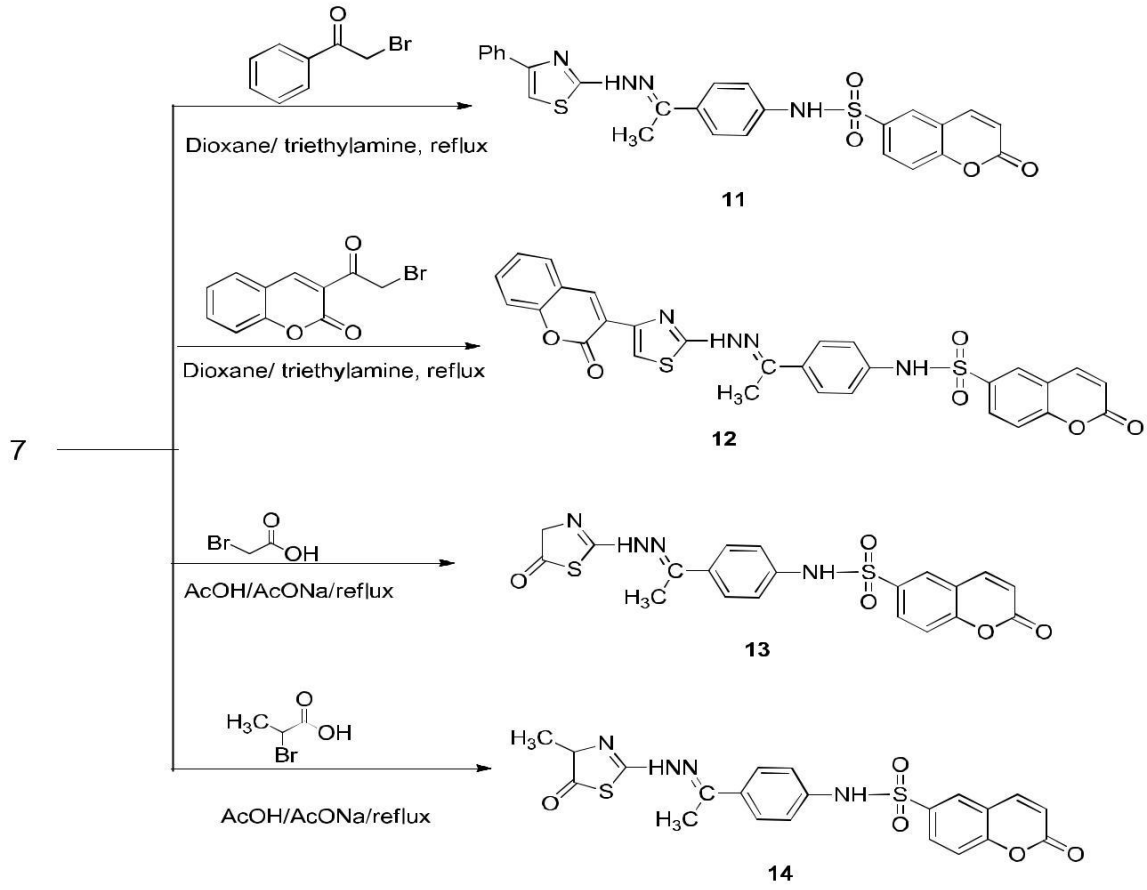
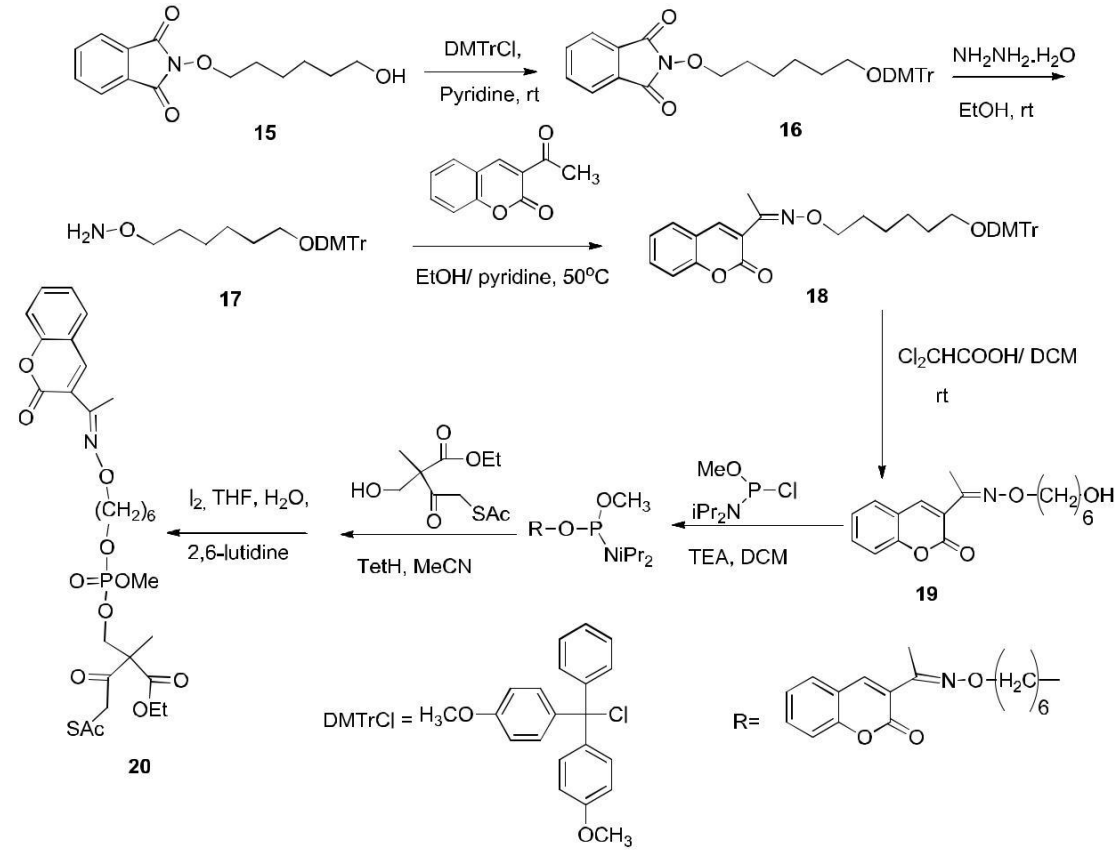
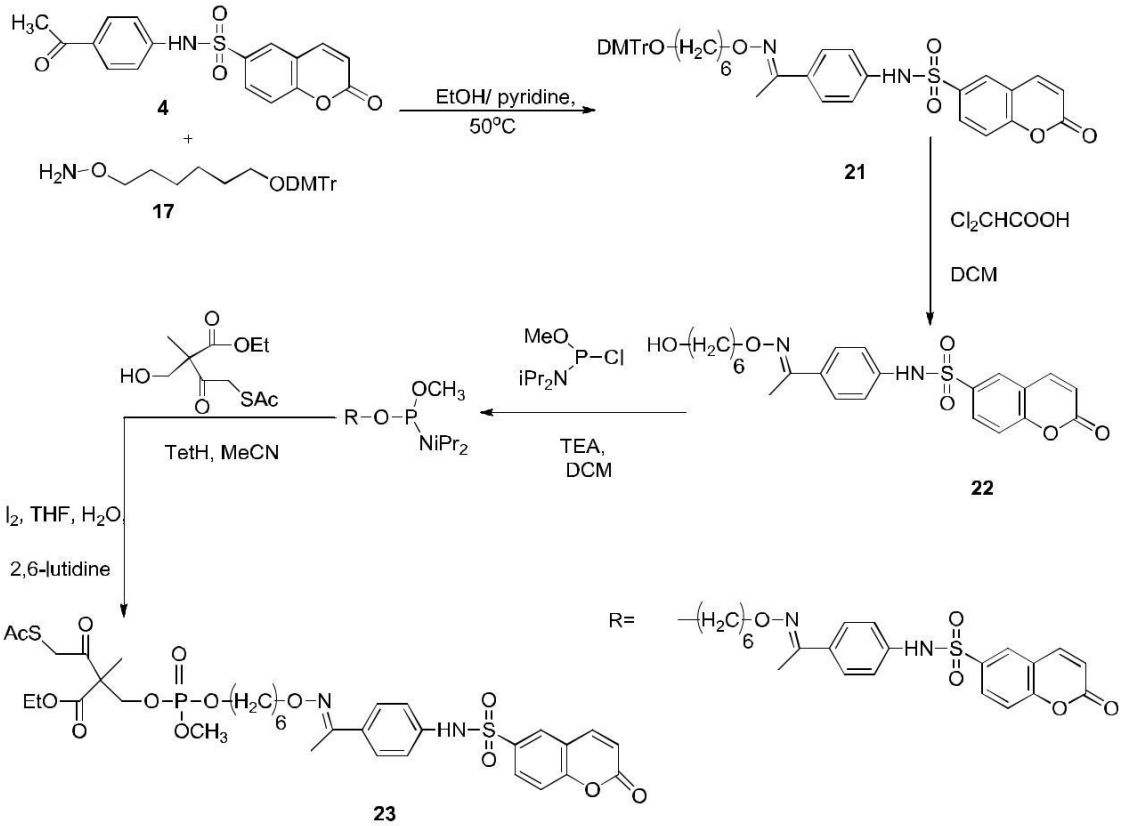


Scheme 4



Scheme 5



Scheme 6