Design and Synthesis of Acridine-4-Carboxamide and Acridine-4- Carboxylate Derivatives as Tyrosine Kinase Inhibitors

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Abstract: Acridine and quinazoline derivatives represent important classes for the treatment of cancer. Many derivatives of them found to be tyrosine kinase inhibitors. In this work novel eight acridine-4-carboxamide and acridine-4- carboxylate derivatives were synthesized from quinazoline and acridine scaffolds. Six of the newly synthesized compounds were chosen by NCI for screening as anticancer. The activity of six compounds (**8a-d, 9a** and **9d**) was tested using the national cancer institute NCI disease oriented antitumor screen protocol. Compound **8c** was proved to be the most active member in this study. This acridine analog **8c** could be considered as useful template for further development to obtain more potent antitumor agents.
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Key words: Acridine-4-carboxamide, acridine-4- carboxylate, quinazoline, kinase inhibitors.

1. Introduction

Protein tyrosine kinases are enzymes involved in many cellular process such as proliferation, metabolism and apoptosis [1]. Several protein kinases are known to be activated in cancer cells [2]. Blocking tyrosine kinase activity therefore represents a rational approach to cancer therapy. Acridines and their analogs represent an important class for treatment of cancer via different mechanisms. They inhibit cancer cells by inhibition of different enzymes such as topoisomerase II [3], telomerase [4] or kinase [5]. They also intercalate with DNA [6]. Many acridine-4-carboxamide derivatives show activity on different cancer cell lines [6]. In recent years, combination chemotherapy with different mechanisms of action is one of the methods that are being adopted to treat cancer. Therefore a single molecule containing more than one pharmacophore, each with different mode of action could be beneficial for the treatment of cancer [7]. Enlightened by the reported activity of quinazoline derivatives as kinase inhibitors [8, 9] and as topoisomerase inhibitors [10], we combine both acridine and quinazoline moieties to produce more potent derivatives. In combining both moieties, different spacers were used. Such spacers were chosen to provide rigid structure and free rotation through phenyl ring in compounds **8a-d** and ethylene bridge in compounds **9a-d** respectively.

2. Experimental

Chemistry

All melting points are uncorrected and determined by the open capillary method using Gallenkamp melting point apparatus (MFB-595-010M; Weiss Gallenkamp, London, UK). IR spectra were recorded on a Shimadzu 435 Spectrometer (IR-435; Shimadzu, Japan) using KBr disks. 1H NMR spectra were recorded on a Perkin-Elmer NMR FXQ-200 MHZ Spectrometer (Tokyo, Japan), using TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX, Mass Spectrometer. Elemental analyses for C, H, and N were within ±0.4% of the theoretical values and were performed at the Microanalytical Center, Cairo University, and they were of the theoretical values. Progress of the reactions was monitored by TLC using precoated aluminum sheets silica gel MERCK 60 F254 (Merck, Germany) and was visualized by UV lamp.

9, 10-Dihydro-9-oxoacridine-4-carbonyl chloride 3

It was prepared by the reported method [11] starting from 9, 10-dihydro-9-oxoacridine-4-carboxylic acid **2** [12] and thionyl chloride. It was used directly for preparation of compounds **8a-d**.

General method for preparation of 2,3-dihydro-3-subistituted -2-thioquinazolin -4*(1H)*-one 5a-d.

They were prepared according to the reported method from reaction of anthranilic acid **4** and different isothiocyanate in ethanol [13]

General method for preparation of 1-(4-aminophenyl)-2,3-dihydro-3-subistituted -2-thioquinazolin-4*(1H)*-one 6a-d.

A mixture of **5a-d** (0.01 mol) and *p*-bromoaniline (1.72g, 0.01 mol) was refluxed with sodium methoxide (2.16 g, 0.04 mol) in absolute ethanol (30 mL). The reaction was monitored by TLC. After the reaction was completed, it was filtered and the solvent removed under vacuum. The products were crystallized from ethanol/ ether mixture[14].

General method for preparation of 2,3-dihydro-1-(2-hydroxyethyl)-3-subistituted-2-thioquinazolin-4-*(1H)*-one 7a-d.

A mixture of **5a-d** (0.01 mol), 2-chloroethanol (0.80 g, 0.01 mol) and potassium carbonate (6.90 g, 0.05 mol) was refluxed in dry acetone for 6-7 hours. The reaction mixture was then, filtered, left to cool and the crystals precipitated were filtered and recrystallized from ethanol [15].

General method for preparation of N-[4-(3,4-dihydro-4-oxo-3-subistituted-2-thioquinazolin-1*(2H)*-yl)phenyl]-9,10-dihydro-9-oxoacridine-4-carboxamide 8a-d.

A mixture of **3** (2.57g, 0.01 mol) and the appropriate compound **6a-d** (0.01 mol) was refluxed in methylene chloride (75mL) and triethylamine (1mL) for 3-4 hours. The reaction was then filtered. The solvent was removed under vacuum. The precipitate which was formed was recrystallized from ethanol/ DMF.

N-[4- (3-Butyl- 3,4- dihydro -4- oxo-2-thioquinazoline-1*(2H)*-yl) phenyl]-9,10-dihydro -9-oxoacridine-4-carboxamide 8a.

Yield 60%; mp: 120^{0}C. IR (cm^{-1}):3300(2NH), 1650,1660 (3 CO), ^{1}H-NMR(DMSO-d6) δppm: 0.88 (t,3H,CH_3), 1.31(m,2H,$\underline{CH_2}$-CH_3) , 1.62(m,2H,$\underline{CH_2}$-CH_2-CH_3),4.30 (t,2H,$\underline{CH_2}$-CH_2-CH_2-CH_3), 7.29-7.93 (m,15H,Ar),12.88 (s,2H,2NH).Anal. Calcd. for $C_{32}H_{26}N_4O_3S$ (546.64) : C 70.31, H 4.79 and N 10.25.Found: C 70.25,H 4.80 and N 9.80

N-[4- (3-Benzyl- 3,4- dihydro -4- oxo-2-thioquinazoline-1*(2H)*-yl) phenyl]-9,10-dihydro -9-oxoacridine-4-carboxamide 8b.

Yield 61%; mp: 148 ^{0}C. IR (cm^{-1}) :3350 (2NH), 1720,1639 (3 CO), ^{1}H-NMR(DMSO-d6) δppm:5.53 (s,2H,CH_2),7.18-7.83(m,20H, Ar),8.47 (s,1H,NH), 8.72(s,1H,NH $\underline{NH}$-CO). MS: m/z 580 (M^{+2}, 0.09%) Anal. Calcd. for $C_{35}H_{24}N_4O_3S$(580.66): C 72.40, H 4.17 and N 9.65. Found: C 72.51, H 4.50 and N 9.27.

-N-[4-(3,4-Dihydro-3-methyl -4- oxo-2-thioquinazoline-1*(2H)*-yl) phenyl] 9,10- dihydro -9-oxoacridine-4-carboxamide 8c.

Yield 55%; mp:225^{0}C. IR (cm^{-1}):3360(2NH), 1670 (3 CO), ^{1}H-NMR (DMSO-d6) δppm:2.51 (s,3H,CH_3),7.18-7.99 (m, 15H, Ar),8.13 (s,1H, NH), 8.72 (s, 1H,NH-CO). Anal. Calcd. for $C_{29}H_{20}N_4O_3S$ (504.56): C 69.03, H 4.00 and N 11.10. Found: C68.80, H4.10 and N10.9.

N-[4- (3-(4-Fluorophenyl)-3,4- dihydro -4- oxo-2-thioxoquinazoline-1*(2H)*-yl) phenyl]-9,10-dihydro -9-oxoacridine-4-carboxamide 8d.

Yield 64%; mp: 245 ^{0}C.IR (cm^{-1}):3400(2NH), 1780 (3 CO), ^{1}H-NMR(DMSO-d6) δppm:6.92-7.79 (m ,19H Ar), 8.5 (s,2H, 2 NH). Anal. Calcd. for $C_{34}H_{21}FN_4O_3S$ (584.62): C 69.85, H 3.62 and N 9.58. Found: C 69.89, H 4.10 and N 9.23.

General method for preparation of 2-(3,4-dihydro-4-oxo-3-subistituted-2-thioquinazolin-1 *(2H)*-yl) ethyl-9,10- dihydro-9- oxoacridine -4- carboxylate 9a-d.

Acridone-4-carboxylic acid **2** (2.39g,0.01 mol) was dissolved in dichloromethane/ THF (1:1 mix.)(75mL) and drops of DMF (till complete dissolution). DCC (2.06g, 0.01 mol) is added and stirred for 5 minutes, followed by addition of the appropriate compounds **7a-d** (0.01 mol). The reaction is kept stirring at room temperature over night. The reaction was then filtered. The filtrate was evaporated to dryness under reduced pressure and the precipitate was crystallized from ethanol/DMF.

2-(3-Butyl-3,4-dihydro-4-oxo-2-thioquinazolin-1*(2H)*-yl)ethyl-9,10-dihydro-9- oxoacridine -4-carboxylate 9a.

Yield 50%; mp:226 ^{0}C.IR (cm^{-1}):3350(NH), 1712,1670 (3CO) ^{1}H-NMR($CDCl_3$) δppm: 0.92 (t,3H,CH_3), 1.32 (m, 2H, $\underline{CH_2}$-CH_3), 1.54 (m, 2H,$\underline{CH_2}$-CH_2-CH_3), 2.91(t,2H,$\underline{CH_2}$-CH_2-CH_2-CH_3) , 3.43 (t,2H, N-CH_2) , 4.23 (t,2H,CH_2-O) , 7.21-7.90 (m,11 H, Ar).Ms:m/z 499.58(M^+,25%). Anal. Calcd. for $C_{28}H_{25}N_3O_4S$ (499.58): C 67.32, H 5.04 and N 8.41. Found: C 67.60, H 5.60 and N 8.59.

2-(3-Benzyl-3,4-dihydro-4-oxo-2-thioquinazolin-1*(2H)*-yl)ethyl-9,10-dihydro-9- oxoacridine -4-carboxylate 9b.

Yield 45%; mp: 195^{0}C.IR (cm^{-1}):3400(NH), 1756,1690 (3 CO), ^{1}H-NMR(DMSO-d6) δppm:2.49 (t, 2H, N-CH_2) , 3.03 (t,2H,CH_2-O), 3.73 (s,2H,CH_2-Ph) ,7.21-7.93 (m, 16H,Ar), 8.56 (s,1H,NH). Anal. Calcd. for $C_{31}H_{23}N_3O_4S$ (533.60): C 69.78, H 4.34 and N 7.87. Found: C 69.80, H 4.41 and N 8.30.

2-(3,4-Dihydro-3-methyl-4-oxo-2-thioquinazolin-1*(2H)*-yl)ethyl-9,10-dihydro -9- oxoacridine -4-carboxylate 9c.

Yield 69%; mp:233^{0}C .IR (cm^{-1}):3350(NH), 1694,1650 (3 CO), ^{1}H-NMR($CDCl_3$) δppm: 2.88(

s,3H,CH_3), 3.62(t,2H,CH_2-N), 3.67(t,2H,CH_2-O),7.21-7.93 (m,11H,Ar). Anal. Calcd. for $C_{25}H_{19}N_3O_4S$ (457.50): C 65.63, H 4.19 and N 9.18. Found: C 65.80, H 4.41 and N 8.60.

2-(3-(4-Fluorophenyl)-3,4-dihydro-4-oxo-2-thioxoquinazolin-1*(2H)*-yl)ethyl-9,10-dihydro-9-oxoacridine -4- carboxylate 9d.

Yield 40%; mp:194^{0}C.IR (cm^{-1}):3320(NH), 1670,1630 (3 CO), ^{1}H-NMR($CDCl_3$) δppm: 2.91 (t,2H,CH_2-N), 3.43(t,2H,CH_2-O),7.22-7.97 (m,15H,Ar). Anal. Calcd. for $C_{30}H_{20}FN_3O_4S$ (537.56): C 67.03, H 3.75 and N 7.82. Found: C 67.29, H 3.81 and N 8.30.

Antitumor screening

Under a sterile condition, cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Biocell, CA, USA); 5×10^5 cell/ml was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 0.01 to 100 µM were prepared in phosphate buffer saline. Each compound was initially solubilized in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1% DMSO. Solutions of different concentrations (0.2 ml) were pipetted into separate well of a microtiter tray in duplicate. Cell culture (1.8 ml) containing a cell population of 6×10^4 cells/ml was pipetted into each well. Controls, containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 ^{0}C. The incubator was supplied with 5% CO_2 atmosphere. After 48 hrs, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution.

3. Results and Discussion

Chemistry

The title compounds were prepared firstly synthesized the tricyclic nucleus acridone-4-carboxylic acid **2** then functionalizing it with quinazoline derivatives. Acridone-4- carboxylic acid **2** was prepared from condensation of anthranilic acid and 2- chlorobenzoic acid to give 1, followed by cyclization according to the reported method [12]. This one was used to prepare intermediate **3** after reacting with thionyl chloride as shown in scheme 1 [11]. Scheme 2 shows preparation of compounds **5a-d.** They were obtained when a mixture of anthranilic acid and different isothiocyanate derivatives were refluxed in ethanol [13]. These compounds later react with p-bromoaniline in presence of sodium methoxide and ethanol and with 2-chloroethanol in presence of potassium carbonate and acetone to produce compounds **6a-d** and **7a-d**, respectively [14, 15]. Acridone-4-carboxamide derivatives were obtained when intermediate **3** was reacted directly with compounds **6a-d** in methylene chloride in presence of triethylamine. Esterification of acridone-4-carboxylic acid **2** with compounds **7a-d** in presence of N,N-dicyclohexyl carbodiimide (DCC) leads to formation of acridone-4-carboxylate derivatives **9a-d** as shown in scheme 3.

Biological activity

The synthesized compounds **8a-d**, **9a** and **9d** were subjected to the NCI in vitro one dose primary anticancer assay using a 3 cell line panel consisting of MCF-7(breast), NCI-H460 (lung) and SF-268 (CNS) cancers. Compounds which reduce the growth of any one of the cell lines to 32% or less passed for evaluation in the full panel of 60 cell lines over a 5-log dose range [16]. Growth percent was shown for each cell line using the known drug 5-fluorouracil (5FU) as positive control.

Conclusion

All compounds show specificity in their action especially on UO-31 cell line (renal cancer) with percent growth range from 85%-63%. The most active one was compound **8c**, while the least active one was compound **9a**. Compound **8c** also show slight activity against SNB-75(CNC) with growth percent equal to 68%. In general compounds **8a-d** were more active than **9a-d**. This may be attributed to the rigidity of structure in compounds **8a-d** exhibited by the presence of phenyl ring as spacer between acridone and quinazoline moieties rather than the ethylene bridge in compounds **9a-d**. This could be considered as useful model for further improvement of activity.

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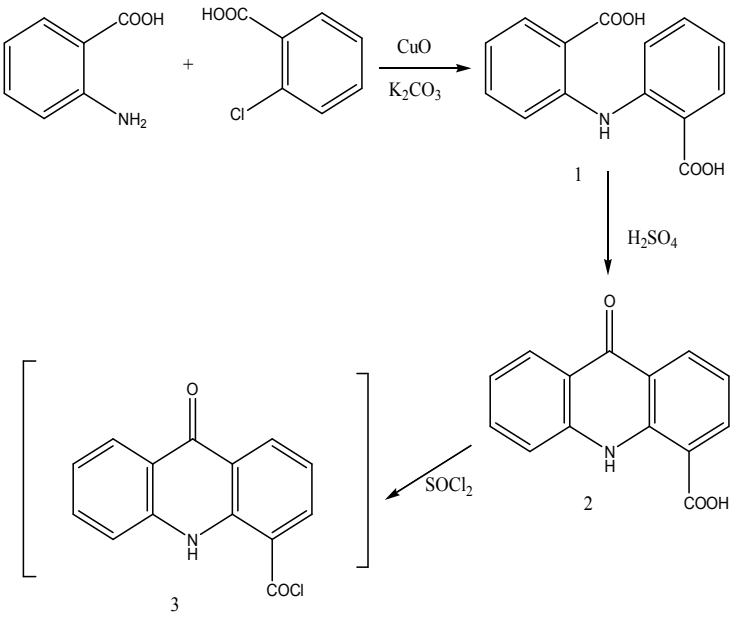
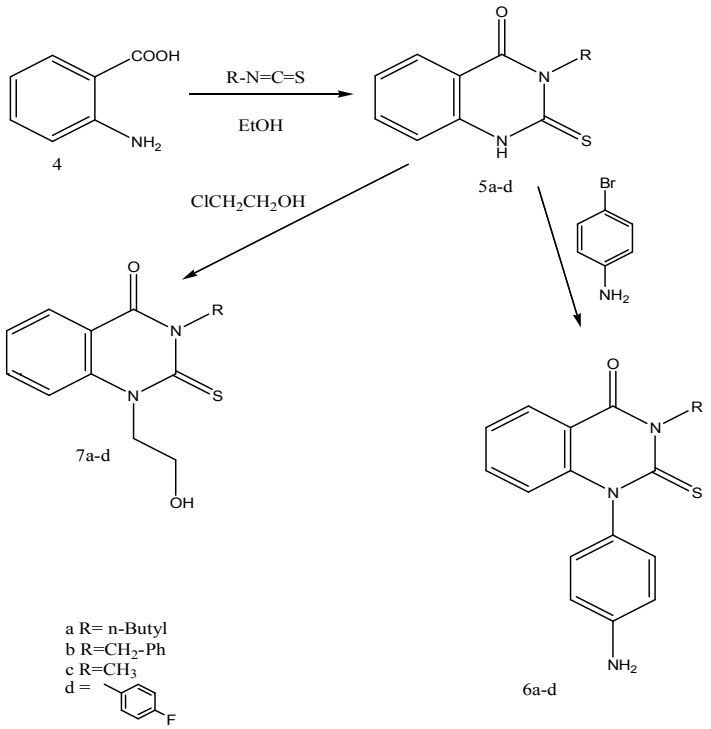
Scheme 1

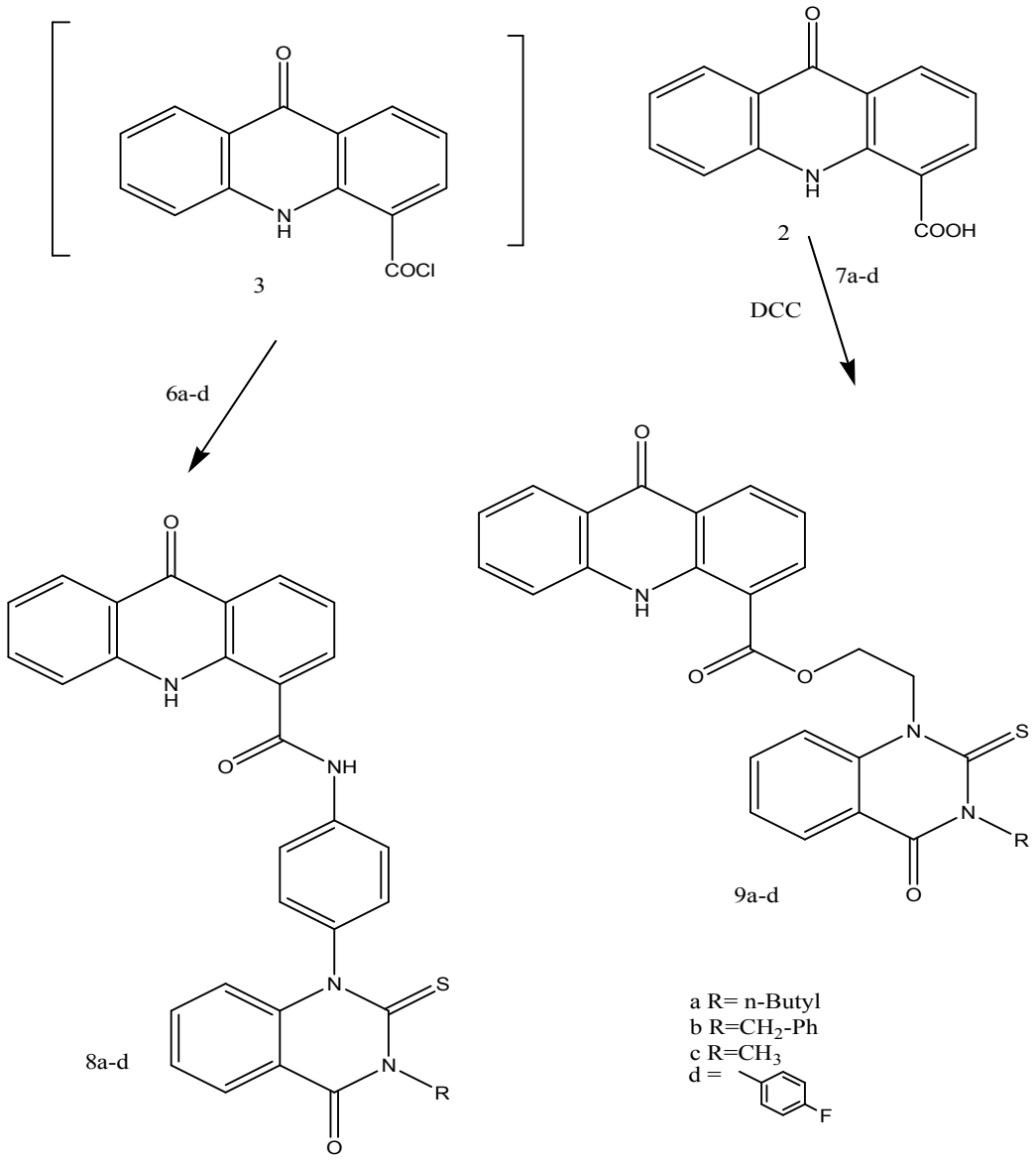

COOH
NH2
+
HOOC
Cl
CuO
K2CO3
COOH
N
H
COOH
1
H2SO4
O
N
H
COOH
2
SOCl2
O
N
H
COCl
3

Scheme 2

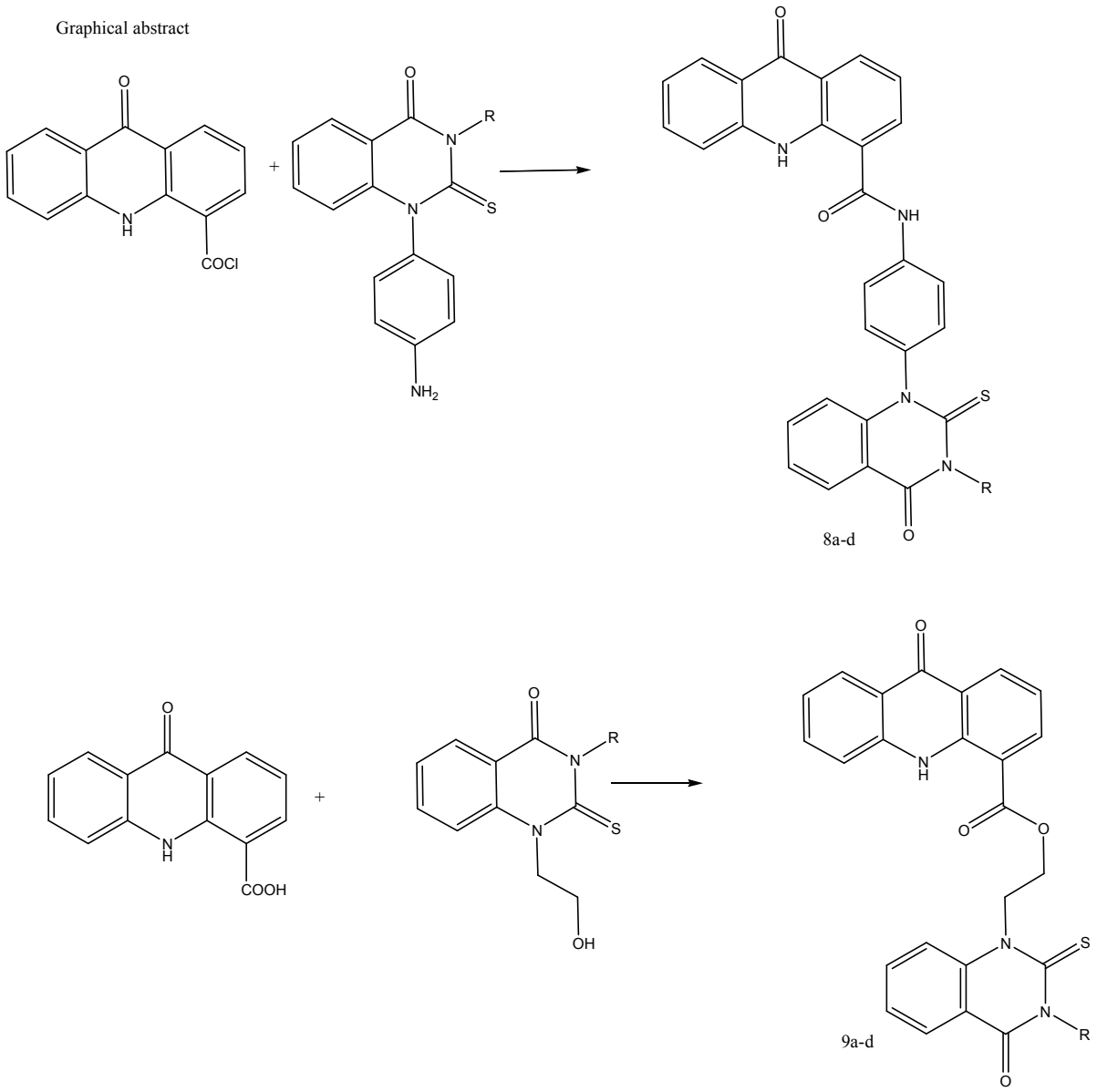


a R= n-Butyl
b R=CH_2-Ph
c R=CH_3
d =

Scheme 3



Graphical abstract



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