Computer Aided Design, Synthesis and Biological Evaluation of Novel Acridine Derivatives a Topoisomerase I Inhibitors

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Abstract: A series of novel 9- anilinoacridines was designed and their molecular docking studies into the active site were examined as topoisomerase I inhibitor. Several compounds showed significant high simulation docking score. The designed compounds were synthesized and biologically evaluated against mammary carcinoma cell line (MCF-7), where compounds 8,11e,11f,13b,14b,14e and 14f showed significant inhibitory activity at a concentration 10µg/mL). It appears that the *in vitro* activity of compounds 8,11e,11f,13b,14b,14e and 14f were consistent with their molecular modeling results, and compound 14b showed the highest activity with IC_{50} value of 7.8 µg. [Journal of American Science. 2010;6(11):148-158]. (ISSN: 1545-1003).

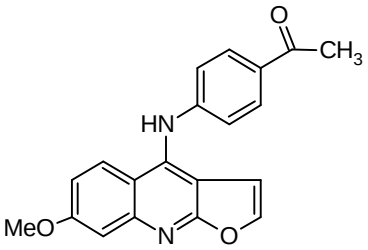
Keywords: Molecular docking, Acridine derivatives, Antitumor

1. Introduction

Topoisomerase-targeting agents that stabilize the cleavable complex formed between the enzyme and DNA have proved to be effective in the treatment of cancer [1]. Topoisomerases are nuclear enzymes; there are two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2) based upon differences in their initial mechanisms wherein a single- or double-stranded DNA break is implicated [2-4].Topoisomerase I participates in the control of the topological state of DNA, and as such this enzyme is essential for DNA transcription and replication as well as other vital processes including chromosome condensation/opening and mitosis [5, 6]. All topoisomerases act through a conserved active-site tyrosine residue to cleave the phosphodiester backbone and form a covalent phosphotyrosine intermediate with the DNA [7]. Human topoisomerase I (Top1) cleaves a single DNA strand through transesterification of Tyr723 and forms a 3`-phosphotyrosine linkage to the DNA. After cleavage, the broken (scissile) DNA strand can rotate around the unbroken (nonscissile) strand and remove DNA supercoils [8]. The enzyme allows both the rewinding of underwound negatively supercoiled DNA and the unwinding of overwound positively supercoiled DNA [9]. The DNA phosphodiester backbone is restored in a second transesterification reaction when the 5`-OH of the broken DNA strand attacks the 3`-phosphotyrosine bond. This religation reaction therefore liberates top1 for subsequent

cleavage/unwinding reactions. Human topoisomerase I (TOP1) is the molecular target of a diverse set of anticancer compounds, including the camptothecins, indolocarbazoles, indenoisoquinolines and 9-anilinoacridines [10].Camptothecin was the first agent identified as a TOP1-targeting agent [11].Irinotecan and topotecan are the only current Top1 inhibitors approved by the Food and Drug Administration (FDA) for the treatment of cancer, and they validate Top1 as a therapeutic target for anticancer drug development. However, these camptothecin derivatives are not ideal drug molecules. These compounds bind to a transient TOP1-DNA covalent complex and inhibit the resealing of a single-strand nick that the enzyme creates to relieve super helical tension in duplex DNA[12]. On the other hand, acridines are known to posses antitumor activity. They exert their antitumor activity through DNA intercalation, [13] inhibition of topoisomerase enzymes [14,15] or inhibition of telomerase[16]. 9-Anilinoacridines is an important class which attracted considerable attention as DNA intercalators[17]. Also, some acridines as the acridine derivative 3-(9-acridinylamino)-5-(hydroxymethyl) aniline (AHMA) were proved to be potent topoisomerase inhibitor[18]. Moreover, some methoxy derivatives of 4-anilinofuro [2,3-b]quinoline I (Fig.1), a bioisotere of 9-anilinoacridines, have been shown to exhibit excellent cytotoxity against cancer cells [19]. Also, methoxy 2-phenylquinoline derivatives, another bioisosters of the acridine ring, as compound II (Fig.1) were found to be active against the growth of certain solid cancer such as NCI-H226 non small cell lung cancer, MDA-MB-231/ATCC breast cancer and Sf-295 CNS cancer [20]. Concerning the antitumor activity

many pyridine-2-one and 3-cyano-2-imino pyridine derivatives exhibit antiproliferative activity [21, 22]. Based on the pre-mentioned review and the urgent need to develop new potential antitumor agents, our current investigation is based on optimization of lead compound by molecular docking studies, using the enzyme bound crystal structure of the Top1 inhibitor topotecan (III)(Fig.1). This involves the synthesis of new substituted acridines such as 9-anilinoacridine; 9-anilinonitroacridines or 9-anilinomethoxy acridines combined to 4-aryl-3-cyanopyridine-6-yl-2-one or 4-aryl-3-cyanopyridine-2-imino-6-yl in order to combine the antitumor activity of both moieties aiming to increase the potency of the resulting hybrid compounds.



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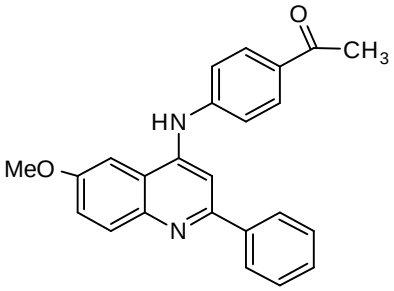
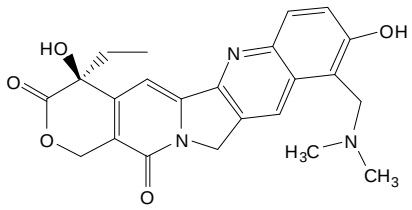


II



III (Topotecan)

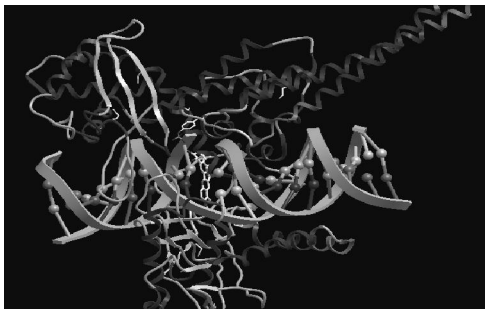


Figure 1. TopI inhibitors and the crystal structure of compound III with the TopI complex

In this work, molecular modeling simulation studies were performed in order to predict the biological activity of the proposed compounds. Docking Study using Molsoft ICM software was performed [23]. The crystal structure of Topotecan/topoisomerase I (Fig. 1) was obtained from protein data bank website (pdb). This regularized protein complex structure was used in determination of the active site that is mentioned in the literature. Docking process was carried out for the test set of compounds (10a-f -12a-f and 13a-f – 15a-f) using the enzyme-ligand interaction energy as scoring function [24].

2. Materials and Methods

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 F 254(Merck,Germany) and was visualized by UV lamp.

2.1. Chemistry

Diphenylamine-2-carboxylic acid 1 and 5-Nitro-diphenylamine-2-carboxylic acid 2 [25].

In this work, compounds 1 and 2 were prepared using "Ulmann reaction" via reaction of o-chlorobenzoic acid or its derivatives and aniline according to the reported method.

4'-Methoxydiphenylamine-2-carboxylic acid 3 [25].

It was prepared according to "Ulmann reaction" using o-chlorobenzoic acid and p-anisidine as reported.

9-Chloroacridine 4, 9-Chloro-3-nitroacridine 5 and 9-Chloro-2-methoxyacridine 6 [26].

They were prepared by reaction of compounds 1-3 with phosphorus oxychloride according to the reported method.

9-(4-Acetylanilino) acridine 7, 9-(4-Acetylanilino)-3-nitroacridine 8 and 9- (4- Acetylanilino)-2-methoxyacridine 9.

A mixture of 4, 5, or 6 (0.04 mol) and p-aminoacetophenone (5.40g, 0.04 mol) was dissolved in DMF (9 mL) and piperidine (2 drops). The mixture was refluxed for 3 hours. The formed precipitate was filtered and crystallized from ethanol. compound 8 Yield 75%; m.p. >300 ^{o}C.MS: m/z (%): 357 [M+](0.5) Anal. Calcd. for $C_{21}H_{15}N_3O_3$(357): C 70.58, H 4.20, N 11.76 Found C 70.34, H 4.13, N 11.76.

General procedure for preparation of 9-[p-(4-Aryl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine derivatives 10a-f, 11a-f and 12a-f.

A mixture of 7, 8 or 9 (0.01 mol), ethylcyanoacetate(1.20 g, 0.01mol), the appropriate aldehyde (0.01mol) and ammonium acetate (6.19g,0.08mol) in n-butanol (30mL) was refluxed for 7 hours. The solid separated on cooling was filtered, dried and crystallized from dimethylformamide and water to provide desired compoundes. 10a-f, 11a-f and 12a-f.

9-[p-(4-Phenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10a

Yield:54%;mp:195-197^{0}C. IR(cm^{-1}):3330 (NHs), 2215(CN), 1660 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.20-8.40(m,18,ArH), 12.20(s, 2H, 2NH) exchanged with D_2O. Anal. Calcd. for $C_{31}H_{20}N_4O$(464): C,80.17;H, 4.31; N, 12.06. Found:C,80.20;H,4.40;N,12.65.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10b

Yield:65%;mp:222-224^{0}C. IR(cm^{-1}):3300 (NHs), 2210 (CN), 1665 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.20-8.40 (m, 17H, ArH), 11.80(s, 2H, 2NH) exchanged with D_2O. MS: m/z (%)483 [M^+] (62.0). Anal.Calcd.for $C_{31}H_{19}FN_4O$ (482) :C,77.17 ;H,3.94 ;N,11.62 .Found:C,77.20;H, 4.40 ;N,11.55.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10c

Yield:63%;mp:215-217^{0}C. IR(cm^{-1}):3310 (NHs), 2220 (CN), 1670 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm:7.20-8.30 (m,17H,ArH),11.60(s,2H,2NH) exchanged with D_2O. Anal.Calcd.for $C_{31}H_{19}ClN_4O$(498.5): C,74.62 ;H,3.81;N,11.23 .Found: C,74.50;H,4.00;N,10.95.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino] acridine 10d

Yield:68%;mp193-196:^{0}C. IR(cm^{-1}):3409(OH), 3332-3239 (NHs), 2200 (CN), 1650 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 6.80-8.60(m, 17H, ArH), 11.50(s, 2H, 2NH) exchanged with D_2O, 12.21(s, 1H, OH) exchanged with D_2O. MS: m/z(%) 480 [M^+] (1.70). Anal.Calcd.for $C_{31}H_{20}N_4O_2$(480) :C , 77.50 ;H, 4.16 ;N,11.66 .Found:C,77.34;H,4.05;N,11.55.

9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 10e

Yield:70%;mp:257-260^{0}C. IR(cm^{-1}):3420-3233 (NHs), 2214 (CN), 1716 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.90 (s,3H,OCH_3),7.20-8.60 (m,17H,ArH),11.8(s,2H,2NH) exchanged with D_2O. Anal.Calcd.for $C_{32}H_{22}N_4O_2$ (494):C, 77.73;H,4.45;N,11.33.Found:C,77.53;H,4.40;N,11.38.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino] acridine 10f

Yield:68%;mp:268-270^{0}C. IR (cm^{-1}):3335-3201(NHs), 2192(CN), 1659(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.70 (s,6H,$2CH_3$) , 7.40-8.60 (m,17H,ArH) ,11.00 (s,2H,2NH) exchanged with D_2O. MS:m/z(%) 508 [M^+] (50.0). Anal.Calcd.for $C_{33}H_{25}N_5O$ (507):C,78.10 ;H,4.93 ;N,13.80 .Found:C,78.10 ;H,5.00;N,13.65.

3-Nitro-9-[p-(4-phenyl-3-cyano-2(1H) oxopyridin-6-yl) anilino] acridine 11a

Yield:60%;mp:>300^{0}C. IR(cm^{-1}): 3447-3231(NHs), 2218(CN), 1731(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.64-8.65 (m,17H,ArH),12.21 (s,2H,2NH) exchanged with D_2O. MS:m/z (%)508 [M^{-1}] (4.15). Anal. Calcd. for $C_{31}H_{19}N_5O_3$(509):C,73.08;H,3.73;N,13.75.Found: C,73.00;H,3.90;N,13.54.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino] -3- nitro acridine 11b

Yield:68%;mp: >300^{0}C. IR(cm^{-1}): 3300 (NHs), 2200 (CN), 1660 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.42-8.62 (m,16H,ArH), 12.00 (s,2H,2NH) exchanged with D_2O. Anal.Calcd.for $C_{31}H_{18}FN_5O_3$ (527):C,70.59

;H,3.41 ;N,13.28 .Found:C,70.40;H,3.50;N,13.35.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitro acridine 11c

Yield:75%;mp: >300^{0}C. IR(cm^{-1}):3404-3330(NHs), 2213(CN), 1650(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.20-8.60 (m, 16H, ArH), 12.20(s, 2H, 2NH) exchanged with D_2O. MS: m/z (%)543.5[M$^+$] (1.34). Anal.Calcd.for $C_{31}H_{18}ClN_5O_3$ (543.5): C,68.44; H,3.31; N,12.88.Found: C,68.16; H,3.72; N,13.01.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitroacridine 11d

Yield:69%;mp: >300^{0}C. IR(cm^{-1}): 3401(OH), 3334-3245(NHs), 2220(CN), 1650(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 6.80-8.60(m, 16H, ArH), 11.60(s, 1H, OH) exchanged with D_2O. 12.20(s, 2H, 2NH) exchanged with D_2O. Anal.Calcd.for $C_{31}H_{19}N_5O_4$ (525):C,70.86;H,3.62;N,13.33.Found: C,70.50; H,3.70;N,13.35.

9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitro acridine 11e

Yield:70%;mp: >300^{0}C. IR(cm^{-1}): 3320 (NHs), 2220(CN), 1710 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm:4.00 (s, 3H, OCH_3), 7.20-8.60 (m, 16H, ArH), 12.20(s, 2H, 2NH) exchanged with D_2O. MS: m/z(%) 538[M^{-1}] (1.34). Anal.Calcd.for $C_{32}H_{21}N_5O_4$ (539):C,71.24;H,3.90;N,12.99. Found: C,70.99 ;H,4.02;N,12.82.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-3-nitroacridine 11f

Yield:65%;mp: >300^{0}C. IR(cm^{-1}): 3330 (NHs), 2200 (CN), 1660 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm:3.40 (s,6H,2CH_3), 7.82-8.40 (m,16H,ArH), 12.00 (s,2H,2NH) exchanged with D_2O. MS:m/z (%)551 [M^{-1}] (0.53). Anal.Calcd.for $C_{33}H_{24}N_6O_3$ (552): C,71.74;H,4.35;N,15.22. Found:C,71.55 ; H,4.40 ;N,15.19.

2-Methoxy-9-[p-(4-phenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 12a

Yield:65%;mp:195-197^{0}C. IR(cm^{-1}):3331(NHs), 2225(CN), 1718(CO). ^{1}H-NMR(DMSO-d_6)δ ppm :3.80 (s, 3H, OCH_3), 7.00-8.10 (m, 17H, ArH), 12.22(s, 2H, 2NH) exchanged with D_2O. MS: m/z (%)494 [M$^+$] (0.26). Anal.Calcd.for$C_{32}H_{22}N_4O_2$(494): C,77.73; H,4.45;N, 11.34 .Found: C,77.69 ;H,4.40;N,11.42.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino]-2-methoxy acridine 12b

Yield:69%;mp:218-220^{0}C. IR(cm^{-1}):3320 (NHs), 2215 (CN), 1665(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.80 (s, 3H, OCH_3), 7.00-8.40 (m, 16H, ArH), 12.00(s, 2H, 2NH) exchanged with D_2O. Anal.Calcd.for $C_{32}H_{21}FN_4O_2$(512) :C,75.00; H,4.10;N,10.99.Found:C,75.10;H,4.00;N,11.40.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino]-2-methoxy acridine 12c

Yield:70%;mp:183-185^{0}C. IR(cm^{-1}): 3350 (NHs), 2210 (CN), 1650 (CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.61 (s,3H, OCH_3), 7.21-8.42 (m,16H,ArH),12 (s,2H,2NH) exchanged with D_2O. Anal. Calcd.for $C_{32}H_{21}ClN_4O_2$ (528.5) :C,72.65 ;H,3.97; N,10.60. Found:C,72.70; H,4.00;N,10.24.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-2-methoxy acridine 12d

Yield:62%;mp:160-162^{0}C. IR(cm^{-1}):3408(OH), 3334-3246(NHs), 2215(CN), 1651(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.41 (s,3H,OCH_3),6.91-8.42 (m,16H,ArH),11.44 (s,1H,OH) exchanged with D_2O, 12.10 (s,2H,2NH) exchanged with D_2O. Anal. Calcd.for $C_{32}H_{22}N_4O_3$(510) :C,75.29 ;H,4.31 ;N,10.98 .Found:C,75.30;H,4.30;N,10.98.

2-Methoxy-9-[p-(4-p-methoxyphenyl-3-cyano-2(1H)-oxopyridin-6-yl) anilino] acridine 12e

Yield:70%;mp:240-242^{0}C. IR(cm^{-1}):3400-3232(NHs),2200(CN), 1722(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.70 (s, 3H, OCH_3), 4.10(s, 3H, OCH_3), 7.20-8.40 (m,16H, ArH), 11.8(s, 2H, 2NH) exchanged with D_2O. MS: m/z (%)524 [M$^+$] (0.28). Anal.Calcd.for $C_{33}H_{24}N_4O_3$ (524): C,75.57;H,4.58;N,10.69 .Found: C,75.40 ;H,4.60;N,11.10.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-oxopyridin-6-yl)anilino]-2-methoxyacridine 12f

Yield: 68%; mp:218-220^{0}C. IR(cm^{-1}): 3340-3400(NHs), 2200(CN),1665(CO). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.86 (s, 6H, 2CH_3), 4.12(s, 3H, OCH_3), 7.54-8.65 (m,16H, ArH), 11.8(s, 2H, 2NH) exchanged with D_2O. MS: m/z (%)537 [M$^+$] (0.13). Anal.Calcd.for $C_{34}H_{27}N_5O_2$ (537): C,75.98; H,5.03; N,13.04. Found: C,75.59; H,5.00; N,13.03.

9-[p-(4-Aryl -3- cyano-2(1H)-iminopyridin-6-yl) anilino] acridine derivatives 13a-f, 14a-f and 15a-f.

A mixture of 7, 8 or 9 (0.01 mol), malononitrile (0.65g, 0.01mol), the appropriate aldehyde (0.01mol) and ammonium acetate (6.19g, 0.08mol) in n-butanol (30mL) was refluxed for 7 hours. The solid separated on

cooling was filtered, dried and crystallized from dimethylformamide and water.

9-[p-(4-Phenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13a.

Yield:66%;mp:270-272 0C. IR(cm-1): 3400-3300(NHs),2215(CN). 1H-NMR(DMSO-d6)δ ppm: 7.21-8.42 (m,18H,ArH), 11.80 (s, 3H, 3NH) exchanged with D2O. Anal.Calcd. for $C_{31}H_{21}N_5$(463): C,80.35; H,4.54; N,15.12. Found: C,80.10; H,4.60; N,15.72.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13b.

Yield: 68%; mp: 219-220 0C. IR(cm-1): 3450-3220 (NHs), 2210 (CN). 1H-NMR(DMSO-d6)δ ppm: 7.22-8.41 (m, 17H, ArH), 11.75 (s, 3H, 3NH) exchanged with D2O. Anal.Calcd. for $C_{31}H_{20}FN_5$ (481): C,77.34; H,4.15; N,14.55. Found: C,77.38; H,4.33; N,14.44.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13c.

Yield:64%;mp:278-2800C. IR(cm-1): 3500-3215(NHs),2225(CN). 1H-NMR(DMSO-d6)δ ppm: 7.43-8.54(m,17H,ArH),11.50(s,3H,3NH) exchanged with D2O. MS: m/z (%)497[M+] (0.1). Anal.Calcd.for $C_{31}H_{20}ClN_5$ (497.5) :C,74.77; H,4.02; N,14.07.Found:C,74.80;H,4.10;N,14.15.

9- [p- (4-p-Hydroxyphenyl -3- cyano- 2 (1H) – iminopyridin -6 -yl) anilino] acridine 13d.

Yield:70%;mp:248-250 ^{0}C. IR(cm^{-1}): 3455(OH), 3300-3224 (NHs), 2209 (CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.44-8.22(m,17H,ArH) ,9.50 (s,1H,OH) exchanged with D_2O , 11.82(s,3H,3NH) exchanged with D_2O. MS: m/z (%)480[M^+] (0.27). Anal.Calcd.for $C_{31}H_{21}N_5O$(479) : C,77.66 ;H,4.38 ;N,14.61.Found:C,77.38;H,4.62;N,14.19.

9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13e.

Yield:65%;mp:235-238 ^{0}C. IR(cm^{-1}): 3421-3200 (NHs), 2220 (CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.41(s,3H,OCH_3) , 7.43-8.51(m,17H,ArH),12.00 (s,3H,3NH) exchanged with D_2O. MS: m/z (%) 493[M^+] (32.61). Anal.Calcd.for $C_{32}H_{23}N_5O$(493): C,77.89 ;H,4.66 ;N,14.20. Found: C,77.90; H,4.60; N,14.20.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 13f.

Yield:72%;mp:271-273^{0}C. IR(cm^{-1}): 3500-3231(NHs), 2216(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.3 (s, 6H, 2CH_3), 6.8-8.6(m, 17H, ArH), 12.2(s, 3H, 3NH) exchanged with D_2O. Anal.Calcd.for $C_{33}H_{26}N_6$ (506) : C,78.26; H,5.14 ; N,16.60. Found: C,78.10; H,4.90; N,16.45.

3-Nitro-9-[p-(4-phenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 14a.

Yield: 65%; mp:>300^{0}C. IR(cm^{-1}): 3500-3225(NHs), 2201(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.20-8.40 (m, 17H, ArH), 12.22(s, 3H, 3NH) exchanged with D_2O. MS: m/z (%) 507 [M^{-1}] (0.03). Anal.Calcd. for $C_{31}H_{20}N_6O_2$ (508) : C,73.23; H,3.94; N,16.53. Found:C,73.00; H,4.00; N,16.22.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]3-nitro acridine 14b.

Yield:62%;mp: >300^{0}C. IR(cm^{-1}): 3225(NHs), 2220(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 7.20-8.40(m, 16H, ArH), 12.14(s, 3H, 3NH) exchanged with D_2O. MS: m/z (%) 527[M^+] (0.38). Anal.Calcd.for $C_{31}H_{19}FN_6O_2$ (526): C,70.72 ; H,3.61; N,15.97. Found:C,70.80; H,3.70; N,15.76.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-3-nitro acridine 14c.

Yield:68%; mp: >300^{0}C. IR(cm^{-1}): 3450-3211(NHs), 2209(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 6.81-8.62 (m, 16H, ArH), 12.35(s, 3H, 3NH) exchanged with D_2O. Anal.Calcd. for $C_{31}H_{19}ClN_6O_2$ (542.5): C,68.57 ; H,3.50 ; N,15.48. Found: C,68.89; H,3.40; N,15.31.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-3-nitro acridine 14d.

Yield:73%;mp: >300^{0}C. IR(cm^{-1}): 3450(OH),3320-3220(NHs),2220(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 6.93-8.44 (m,16H,ArH), 9.91(s,1H,OH) exchanged with D_2O 12.20 (s,3H,3 NH) exchanged with D_2O. Anal.Calcd. for $C_{31}H_{20}N_6O_3$ (524): C,70.99; H,3.82; N,16.03. Found:C,71.35; H,3.89; N,16.03.

9-[p-(4-p-Methoxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-3-nitro acridine 14e.

Yield:70%;mp:>300 ^{0}C. IR(cm^{-1}): 3400-3300 (NHs), 2200 (CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.60 (s,3H,OCH3), 7.22-8.42 (m,16H,ArH), 12.11 (s,3H,3NH) exchanged with D_2O. MS: m/z (%) 537 [M^{-1}] (1.92). Anal.Calcd. for $C_{32}H_{22}N_6O_3$ (538): C,71.37; H,4.09; N,15.61. Found: C,71.30; H,4.26; N,15.31.

9-[p-(4-p-Dimethylaminophenyl -3- cyano-2(1H)-iminopyridin-6-yl) anilino]-3-nitroacridine 14f.

Yield: 68%;mp: >300 ^{0}C. IR(cm^{-1}): 3450-3300 (NHs), 2210 (CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.11 (s, 6H, 2CH_3), 7.21-8.00 (m, 16H, ArH), 12.14(s, 3H, 3NH) exchanged with D_2O. Anal.Calcd. for $C_{33}H_{25}N_7O_2$ (551) : C,71.87; H,4.53; N,17.78. Found:C,71.90; H,4.60; N,17.60.

2-Methoxy-9-[p-(4-phenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino] acridine 15a.

Yield:75%;mp: 228-230^{0}C. IR(cm^{-1}): 3450-3300 (NHs),2220 (CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.75(s,3H,OCH_3), 7.11-8.22 (m,17H,ArH),12.00 (s,3H,3NH) exchanged with D_2O. Anal.Calcd. for $C_{32}H_{23}N_5O$ (493) : C,77.89 ; H,4.66 ; N,14.20. Found: C,77.80; H,4.64; N,14.35.

9-[p-(4-p-Fluorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxyacridine 15b.

Yield:68%;mp:258-260^{0}C. IR(cm^{-1}): 3300 (NHs), 2210 (CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.65(s,3H,OCH3) , 7.32-8.52 (m,16H,ArH),11.95 (s,3H,3NH) exchanged with D_2O. Anal.Calcd.for $C_{32}H_{22}FN_5O$ (511) : C,75.15 ;H,4.30 ;N,13.70 . Found:C,74.95;H,4.45;N,13.91.

9-[p-(4-p-Chlorophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxy acridine 15c.

Yield:72%; mp:230-232^{0}C. IR(cm^{-1}): 3400-3201(NHs), 2225(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.82 (s, 3H, OCH_3), 7.22-8.42 (m, 16H, ArH), 12.35(s, 3H, 3NH) exchanged with D_2O. Anal.Calcd. for $C_{32}H_{22}ClN_5O$ (527.5): C,72.80; H,4.17; N,13.27. Found:C,72.56; H,4.18; N,13.14.

9-[p-(4-p-Hydroxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxyacridine 15d.

Yield:70%; mp: 222-225^{0}C. IR(cm^{-1}): 3450 (OH), 3320(NHs), 2210(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 4.00 (s, 3H, OCH_3), 6.8-8.6 (m, 16H, ArH), 9.6(s, 1H, OH) exchanged with D_2O, 11.73 (s, 3H, 3NH) exchanged with D_2O. MS: m/z (%) 508 [M^{-1}] (3.11). Anal.Calcd. for $C_{32}H_{23}N_5O_2$ (509): C,75.44; H,4.52; N,13.75. Found: C,74.21; H,4.39; N,14.00.

2-Methoxy-9-[p-(4-p-methoxyphenyl-3-cyano-2(1H)-iminopyridin-6-yl) anilino] acridine 15e.

Yield:65%;mp:165-167 ^{0}C. IR(cm^{-1}): 3334-3246(NHs), 2199(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.22(s,3H,OCH_3), 3.92(s,3H,OCH_3) , 6.91-8.43 (m,16H,ArH), 11.95 (s, 3H,3 NH) exchanged with D_2O. MS: m/z (%)523 [M$^+$] (1.75). Anal.Calcd. for $C_{33}H_{25}N_5O_2$ (523): C,75.72; H,4.78; N,13.38. Found:C,75.40; H,4.50; N,13.42.

9-[p-(4-p-Dimethylaminophenyl-3-cyano-2(1H)-iminopyridin-6-yl)anilino]-2-methoxyacridine 15f.

Yield:67%;mp:180-182^{0}C. IR(cm^{-1}): 3400-3300(NHs),2206(CN). ^{1}H-NMR(DMSO-d_6)δ ppm: 3.00 (s, 6H, 2CH_3), 3.9(s, 3H, OCH_3), 6.8-8.6 (m, 16H, ArH), 11.8 (s, 3H, 3NH) exchanged with D_2O. Anal.Calcd. for $C_{34}H_{28}N_6O$ (536) : C,76.12; H,5.22; N,15.67. Found:C,76.20; H,5.30; N,15.53.

2.2. Biological screening

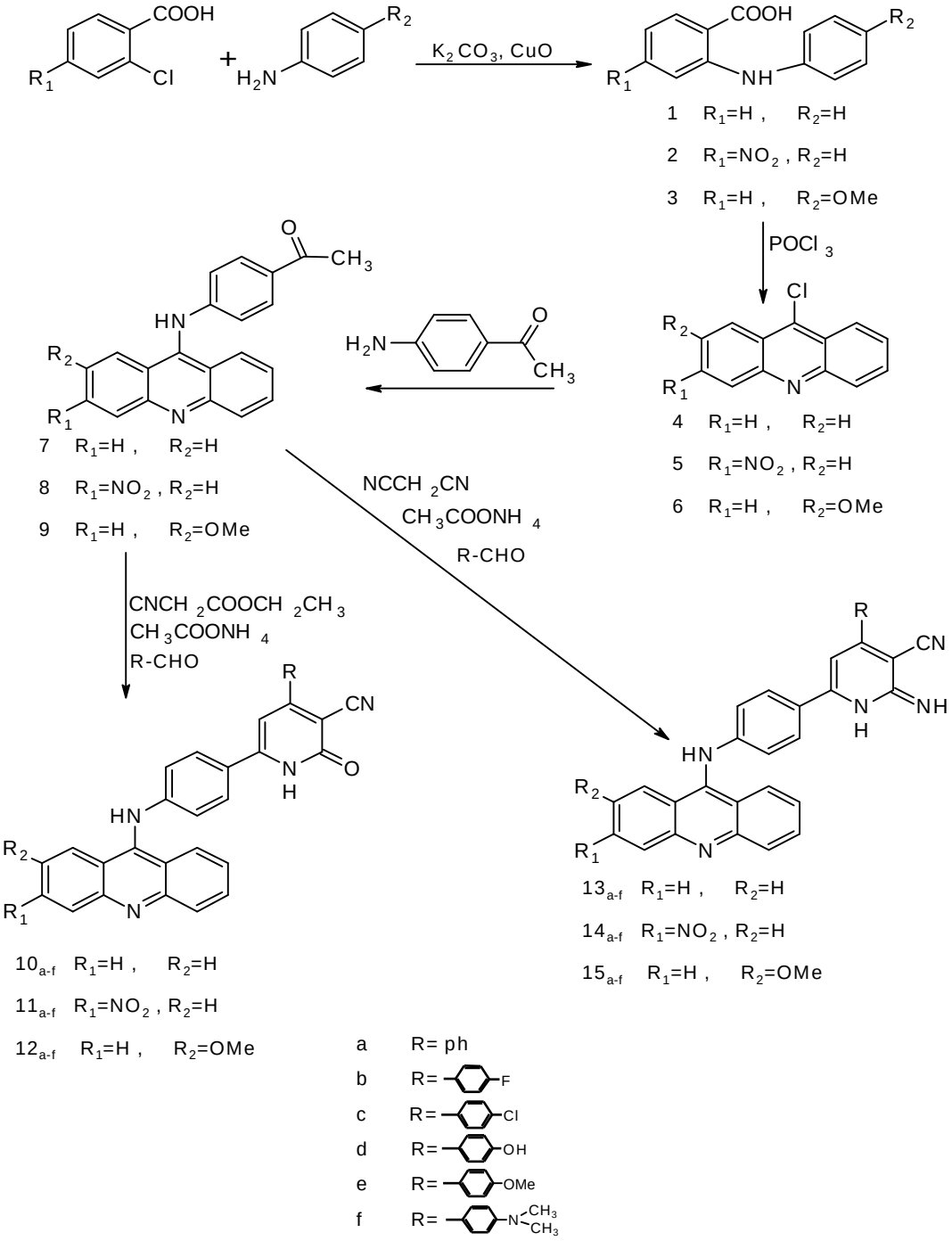
MCF-7 breast cancer cells were plated in 96 multiwell plates (104 cells/well) for 24 hours before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5 and 10 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37 ^{o}C and in atmosphere of 5% CO_2. After 48 hours, cells were fixed, washed and stained with Sulforhodamie B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader.

3. Results and Discussion

3.1. Synthesis.

For the synthesis of the target compounds 10a-f - 15a-f the following straightforward pathway was pursued. Compounds 1-3 were prepared using Ulmann reaction according to the reported method [25]. Compounds 1-3 were used to prepare the intermediate compounds 4-6 respectively according to the reported method [26]. Compounds 7-9 were prepared from compounds 4-6, respectively [27]. The infrared spectrum of the compounds showed reappearance of NH and C=O groups. The final compounds were obtained as shown in scheme 1 using a combinatory chemistry model using multicomponent reaction (MCRs) [28, 29]. This type of reaction is preferred since it is easier to perform, gives higher yield and is less time consuming [30]. The time needed for completing the reaction was monitored by TLC using chloroform: methanol 9.5:0.5. The final compounds were prepared by refluxing an equimolar amount of compounds 7-9 and the appropriate aldehyde in the presence of excess ethylcyanoacetate or malononitrile to afford the corresponding compounds 10a-f -12a-f or compounds 13a-f -15a-f, respectively.

Scheme 1



3.2. Molecular docking studies of the new compounds with topoisomerase I

This technique is considered direct molecular modeling where the 3D structure of the enzyme is known and is used to know the detailed intermolecular interactions between the ligand and the target protein. An automated docking study was carried out using the crystal structure of inhibitor Topotecan/topoisomerase I complex obtained from protein data bank website (pdb) entry 1SC7; having resolution of 2.0 A°. This regularized protein complex structure was used in determination of the enzyme active site that is mentioned in the literature. The performance of

the docking method on topoisomerase I inhibitors was evaluated and validated by re-docking the crystal ligand topotecan where RMSD value obtained was 0.00421. Docking process was carried out for the test set of compounds (10a-f -12a-f and 13a-f – 15a-f).

In the flexible-ligand-rigid enzyme docking, the enzyme was represented by six potential energy maps, namely, electrostatic, hydrogen bond, hydrophobic, and three van der Waals. Interactive docking using Mol table ligand was carried out for all the conformers of each compound of the test set to the selected active site of topoisomerase I. Each docked compound was assigned a score according to its fit in the ligand binding pocket (LBP).

The predicted binding energies of the new compounds are listed in Tables 1&2. The docking poses of Topotecan III and the three coumpounds possessing the lowest binding energies, 14b, 14e and 11e, into the active site of TopI is shown in Fig. 2.

Docking results provided useful information in understanding the structural features of the target and the necessary chemical features of the ligands. This was extended to the successful designing of our acridine derivatives where most analogs were highly active analogs against Top I.

(a)

(b)

(c)

(d)

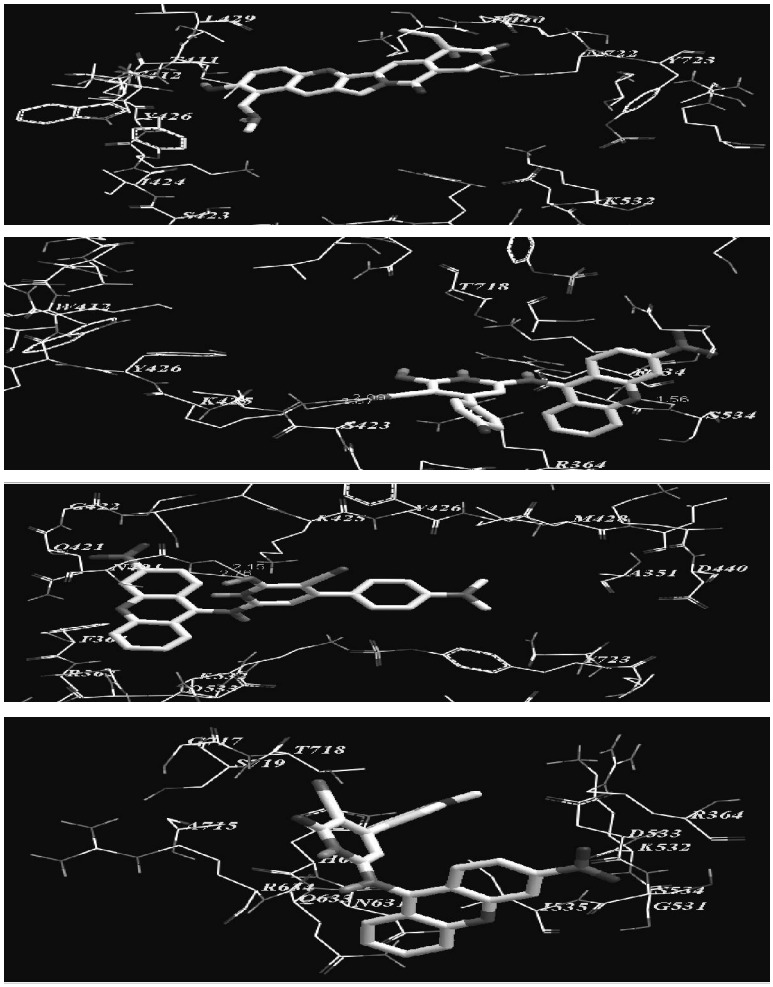


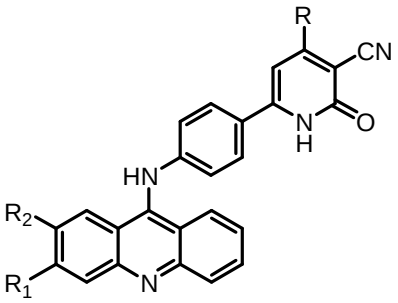
Figure 2; (a), (b), (c) and (d) the proposed binding mode of Topotecan III, compound 14b, 14e and 11e inside the active site of TopI resulting from docking, respectively. The most important amino acids are shown together with their respective numbers. Compound 14b form two hydrogen bonds with Ser423.

3.3. Anticancer screening

Measurement of potential cytotoxity of the new compounds against breast cancer MCF-7 cell line by sulforhodamine B (SRB) assay: Potential cytotoxicity of the compounds against MCF-7 breast cancer cell line was tested using the method of skehan co-worker [31]. The relation between surviving fraction and drug concentration was plotted to get the survival curve for each compound. Also, IC50 for each derivative was determined which is the dose of the compound reduces survival to 50%. Results are shown in Table 3. It can be seen from the data obtained that compounds 8, 11e, 11f, 13b, 14b, 14e and 14f showed activity at a concentration 10 µg or less. The most active one is 14b. Regarding the structure of the active compounds we can conclude that acridine derivatives substituted with nitro group showed higher activity compared to the unsubstituted or methoxy congeners as shown by 11e and 14b.

The p-substituted-3-cyano-2-iminopyridine derivatives exhibited higher antitumor activity than their 2-oxocongeners.

Table 1. Best docking conformer for each compound in the test set (10a-f -12a-f) docked into the active site of Top1.



R	*R_1*	*R_2*	*Cpd No.*	*Docking value (kcal/mol)*	*R*	*R_1*	*R_2*	*Cpd No.*	Docking value (kcal/mol)
Ph	H	H	**10a**	-66.1	p-OH-C_6H_5	NO_2	H	**11d**	-65.3
p-F-C_6H_5	H	H	**10b**	-79.1	p-MeO-C_6H_5	NO_2	H	**11e**	-79.9
p-Cl-C_6H_5	H	H	**10c**	-69.9	p-$N(CH_3)_2$-C_6H_5	NO_2	H	**11f**	-72.7
p-OH-C_6H_5	H	H	**10d**	-63.4	Ph	H	MeO	**12a**	-66.4
p-MeO-C_6H_5	H	H	**10e**	-71.3	p-F-C_6H_5	H	MeO	**12b**	-78.4
p-$N(CH_3)_2$-C_6H_5	H	H	**10f**	-70.6	p-Cl-C_6H_5	H	MeO	**12c**	-72.7
Ph	NO_2	H	**11a**	-66.9	p-OH-C_6H_5	H	MeO	**12d**	-70.4
p-F-C_6H_5	NO_2	H	**11b**	-70.1	p-MeO-C_6H_5	H	MeO	**12e**	-73.1
p-Cl-C_6H_5	NO_2	H	**11c**	-64.1	p-$N(CH_3)_2$ -C_6H_5	H	MeO	**12f**	-75.8

4. Conclusion,

Compounds 8, 11e, 11f, 13b, 14b, 14e and 14f showed activity at a concentration 10 µg or less. The most active one is 14b. Regarding the structure of the active compounds we can conclude that acridine derivatives substituted with nitro group showed higher activity compared to the unsubstituted or methoxy congeners as shown by 11e and 14b. The p-substituted-3-cyano-2-iminopyridine derivatives exhibited higher antitumor activity than their 2-oxocongeners.

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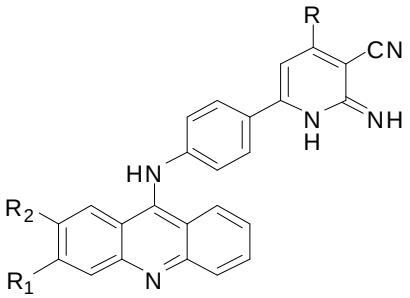

Table 2. Best docking conformer for each compound in the test set (13a-f-15a-f) docked into the active site of Top1.

R	*R_1*	*R_2*	*Cpd No.*	*Docking value (kcal/mol)*	*R*	*R_1*	*R_2*	*Cpd No.*	Docking value (kcal/mol)
Ph	H	H	**13a**	-63.1	p-OH-C_6H_5	NO_2	H	**14d**	-67.3
p-F-C_6H_5	H	H	**13b**	-77.4	p-MeO-C_6H_5	NO_2	H	**14e**	-82.4
p-Cl-C_6H_5	H	H	**13c**	-70.3	p-N$(CH_3)_2$ - C_6H_5	NO_2	H	**14f**	-75.7
p-OH-C_6H_5	H	H	**13d**	-66.6	Ph	H	MeO	**15a**	-67.3
p-MeO-C_6H_5	H	H	**13e**	-73.7	p-F-C_6H_5	H	MeO	**15b**	-78.4
p-N$(CH_3)_2$ - C_6H_5	H	H	**13f**	-72.6	p-Cl-C_6H_5	H	MeO	**15c**	-69.9
Ph	NO_2	H	**14a**	-68.3	p-OH-C_6H_5	H	MeO	**15d**	-71.2
p-F-C_6H_5	NO_2	H	**14b**	-86.8	p-MeO-C_6H_5	H	MeO	**15e**	-74.1
p-Cl-C_6H_5	NO_2	H	**14c**	-73.1	p-N$(CH_3)_2$ - C_6H_5	H	MeO	**15f**	-73.5

Table 3: IC_{50} values of the most active compounds against MCF-7 breast cancer cell line.

Compound	IC_{50} in µg/mL
8	9.23
11e	8.86
11f	10
13b	9.93
14b	7.80
14e	9.73
14f	9.41

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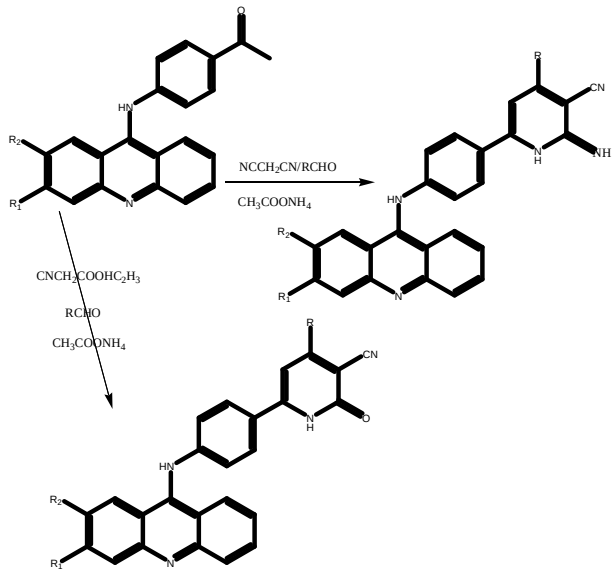
Graphical abstract



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