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# SYNTHESIS AND EVALUATION OF CYTOTOXIC ACTIVITY OF NEW ACENAPHTHO TRIAZIN BENZAMIDE DERIVATIVES

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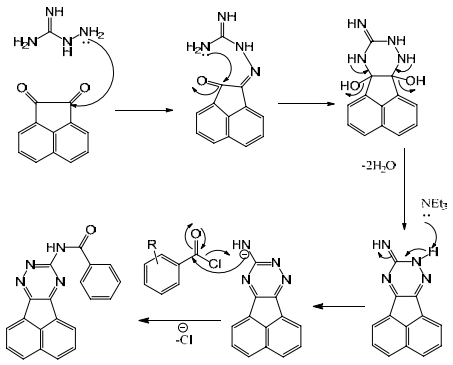
In this paper, we described the facile synthesis of new acenaphtho triazin benzamid derivatives. For this purpose, acenaphtho-(1, 2-e) (1, 2, 4) triazine-9-(8H) imine was prepared with the reaction of acenaphthoquinone and guanidine. Then, the reaction of this product with benzoyl chloride afforded the desired compounds in a good yield. The cytotoxicity of the synthesized compounds was also studied against human cancer cell lines including non-small cell lung (A549), ovarian (SKOV3) and breast (MCF-7) cancer cell lines, as well as normal lung cell line (MRC-5). Among them 5b, 5c and 5h showed moderate to good activity.



## INTRODUCTION

Maximizing synthetic efficiency by designing complexity-generating diversity-oriented synthesis is gaining more and more importance in modern organic chemistry and drug discovery endeavors. In this regard, the development of novel compounds caused higher attention of medicinal and biological chemists.

It is thought that rigid multipart heterocyclic compounds have the prominent role in the development of anticancer agents due to their ability in insertion between stacked base pairs of oligonucleotides and action as an intercalator.

The 1, 2, 4-triazine ring system is characterized as an important heterocyclic core playing a significant role in medicinal chemistry, especially in the field of antimicrobial agents and chemotherapy. 1, 2, 4-triazine derivatives exhibit a broad spectrum of interesting pharmacological and biological properties such as antimicrobials, herbicides, bactericides, fungicides.

Acenaphthene derivatives have gained great importance due to their diverse biological properties including antitumor,[1,2] antifungal,[3] antimicrobial,[4] anti-inflammatory[5,6] and insecticidal[7,8] activities.

On the other hand, synthesis of polycyclic aromatic compounds containing triazine derivatives have attracted widespread attention because of their unique biological and pharmacological properties.[9,10]

In order to obtain novel acenaphthene derivatives with a wide spectrum of pharmaceutical applications, we report herein the synthesis of a series of

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acenaphthene derivatives containing thiazole backbone and the results of their preliminary *in vitro* antitumor evaluation.

In this view, privileged heterocyclic structures have been constructed around the acenaphthene core.[11] Some of the acenaphthene derivatives containing thiazole backbone have been reported as antitumor agents.[12]

In line with our research on the synthesis of bioactive heterocyclic compounds,[13,14] we have developed convenient protocol for the synthesis of new acenaphtho tri azin benzamid derivatives through two step condensation. In the initial step, acenaphthoquinone reacted with guanidine in the presence of tri ethyl amine to formation of acenaphtho[1,2-e]-1,2,4-triazine-9(8H)-imine and subsequent reacted with benzoyl chloride derivatives to formation the target product.

## MATERIAL AND METHODS

High-purity chemical materials were purchased from the Merck Company. Melting points were evaluated by using capillary tubes on an electro thermal digital apparatus and were uncorrected. Synthesized compounds were identified by FT–IR, $^{1}$H-NMR and $^{13}$C-NMR spectra and Elemental analyses. Fourier transform infrared spectra (FT–IR), of the powders as pellets in KBr were recorded using a Fourier transmission infrared spectrometer (Perkin–Elmer BX- II). $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded in DMSO-d6 on Brucker 300 MHz spectrometer (Chemical shifts are given in parts per million or ppm) and Elemental analyses (C, H, N) were performed by the Micro analytical Unit. The purity detection of the products and reaction following were performed by TLC on silica gel PolyGram SILG/UV 254 plates.

### General procedure for the Synthesis of acenaphtho [1,2-e]-1,2,4-triazine-9(8H)-imine (3)

A mixture of acenaphthoquinone **(1)** (10 mmol) and guanidine (2) (10 mmol), and tri ethyl amine (10mmol) was refluxed in EtOH (120 mL) for 24 hr. After completion of the reaction (monitored by TLC), the resulting solid product was recrystallized in hot isopropanol, then it was dried in oven (90°C) to synthesize of the target product (3) (Scheme 1).

***Acenaphtho[1,2-e]-1,2,4-triazine-9(8H)-imine* (3)**

Yield 90%; m.p. 264-266 °C; IR (KBr, cm$^{-1}$): 3334, 3168, 2920,1640, 1583, 766; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 7.78- 8.31 (m, 6H, J = 7.5Hz, H aromatic),7.42 (S, 2H, NH); $^{13}$C-NMR (75 MHz, DMSO-d6) δ: 163, 149, 134, 133, 131, 131, 130, 129,125,121.

### General procedure for the Synthesis of N-(acenaphtho[1,2-e][1,2,4] triazin-9-yl) benzamide (5)

To stirred solution of the formed product in previous section (3) (1 mmol) and benzoyl chloride (4) in ethanol, tri ethyl amine (3mmol) was added drop wise, then the resulting mixture was refluxed until the reaction completed (checked with TLC). The resulting yellow solid product was recrystallized with hot ethanol to give the pure product (5), then the resulted precipitate was completely dried in an electrical oven (Scheme 2). All the compounds are new and characterized by instrumental analysis and their structures are confirmed.

Characteristic data for new synthesized compounds:

***N-(acenaphtho[1,2-e][1,2,4]triazin-9-yl)benzamide* (5a)**

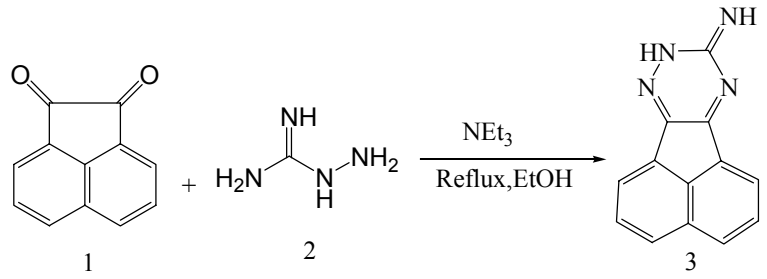
Yield 96%; m.p. 233-234 °C; IR (KBr, cm$^{-1}$): 3325, 3138, 2916,1657, 1591, 768; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 7.78- 8.31 (m, 11H, J = 7.5Hz, H Aromatic),7.42 (S, 2H, NH); $^{13}$C-NMR (75 MHz, DMSO-d6) δ: 163, 159, 149, 134, 133,131, 131, 130, 130, 129,125,121.

***N-(acenaphtho[1,2-e][1,2,4]triazin-9-yl)-2-chlorobenzamid* (5b)**

Yield 92%; m.p. 256-257 °C; IR (KBr, cm$^{-1}$): 3327, 3138, 2918, 1658, 1590, 765; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 7.43- 8.33 (m, 10H, J = 7.5Hz, H Aromatic), 7.43 (S, 2H, NH); $^{13}$C-NMR (75 MHz, DMSO-d6) δ: 163, 159, 149, 133, 131,131,130, 130, 129,125,121.

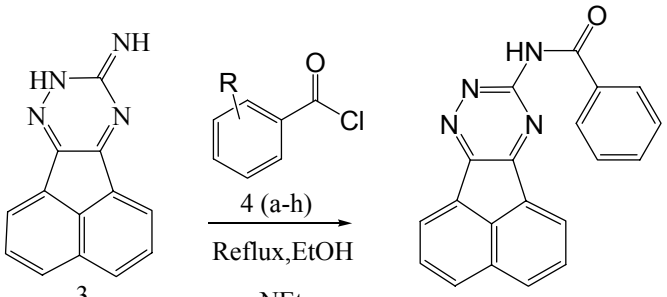
***N-(acenaphtho[1,2-e][1,2,4]triazin-9-yl)-4-chlorobenzamid* (5c)**

Yield 97%; m.p. 260-262 °C; IR (KBr, cm$^{-1}$): 3326, 3132, 2920,1659, 1593, 763; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 7.43- 8.33 (m, 11H, J = 7.5Hz, H Aromatic),7.43 (S, 2H, NH); $^{13}$C-NMR (75 MHz, DMSO-d6) δ:177, 163, 159 , 149, 134, 133, 131,131,130, 130, 129,125,121.




1 2 3

Scheme 1




3 5 (a-h)

Scheme 2

***N-(acenaphtho[1,2-e][1,2,4]triazin-9-yl)-2-bromobenzamid*** **(5d)**

Yield 93%; m.p. 249-250 °C; IR (KBr, $cm^{-1}$): 3327, 3124, 2918,1656, 1580, 767; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 7.43- 8.33 (m, 11H, J = 7.5Hz, H Aromatic),7.43 (S, 2H, NH); $^{13}$C-NMR (75 MHz, DMSO-d6) δ: 163, 159, 149, 133, 131,131,130, 130, 129,125,121.

***N-(acenaphtho[1,2-e][1,2,4]triazin-9-yl)-4-nitrobenzamid*** **(5e)**

Yield 96%; m.p. 232-234 °C; IR (KBr, $cm^{-1}$): 3325, 3128, 2924,1659, 1586, 766; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 7.42- 8.30 (m, 11H, J = 7.5Hz, H Aromatic),7.43 (S, 2H, NH); $^{13}$C-NMR (75 MHz, DMSO-d6) δ: 163, 160, 149, 133, 131, 131, 130, 130, 129, 125, 121.

***N-(2-chlorobenzyl)acenaphtho[1,2-e][1,2,4]triazin-9-amine*** **(5f)**

Yield 89%; m.p. 234-236 °C; IR (KBr, $cm^{-1}$): 3322, 3128, 2927,1656, 1590, 769; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 4.01 (S, 2H), 7.42- 8.33 (m, 11H, J = 7.5Hz, H Aromatic); $^{13}$C-NMR (75 MHz, DMSO-d6) δ: 163, 160, 149, 134, 133, 131, 131, 130, 130, 129, 125, 121, 75.

***N-benzyl acenaphtho[1,2-e][1,2,4]triazin-9-amine*** **(5g)**

Yield 88%; m.p. 233-234 °C; IR (KBr, $cm^{-1}$): 3325, 3138, 2926,1657, 1591, 768; $^{1}$HNMR (300 MHz, DMSO-$d_6$) δ: 4.43 (S, 2H), 7.42-8.16 (m, 11H, Aromatic); $^{13}$C-NMR (75 MHz, DMSO-d6) δ: 163, 159, 149, 133, 133, 131, 131, 131, 130, 130, 129, 125, 121, 53.

## RESULTS AND DISCUSSION

Acenaphto triazin benzamide derivatives were synthesized by two-step condensation. In the first step, acenaphtoquinone (1) reacted with guanidine (2) in the presence of tri ethyl amine and the resulted product was reacted with benzoyl chloride to formed the titled compound (Scheme 3).

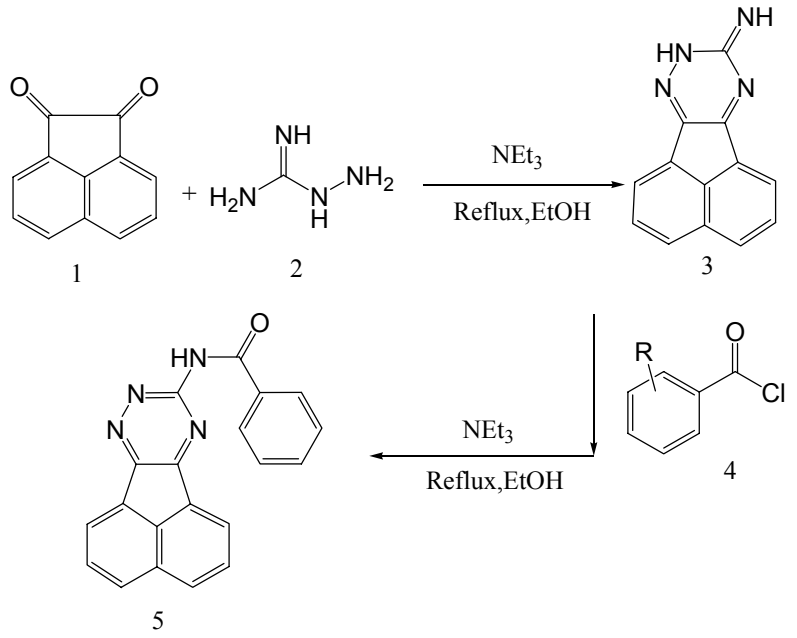
In order to explore the generality of the present synthetic role, various types of benzoyl chloride and benzyl halide were used. The obtained results are presented in Table (1).

The possible mechanism for the two step synthesis of acenaphto triazin benzamide derivatives is described in Scheme 4.

### Biological assay

#### *Cell lines and cell culture*

Human non-small cell lung cancer cell line (A549), human ovarian cancer cell lines (SKOV3) and human breast cancer cell line (MCF-7) as well as normal lung cell line (MRC-5) were obtained from National Cell Bank of Iran (NCBI, Pasteur Institute, Tehran, Iran). A549 and SKOV3 cells were cultured in DMEM medium, MCF-7 in RPMI 1640 media and MRC-5 in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin at 37 °C in humidified $CO_2$ incubator.

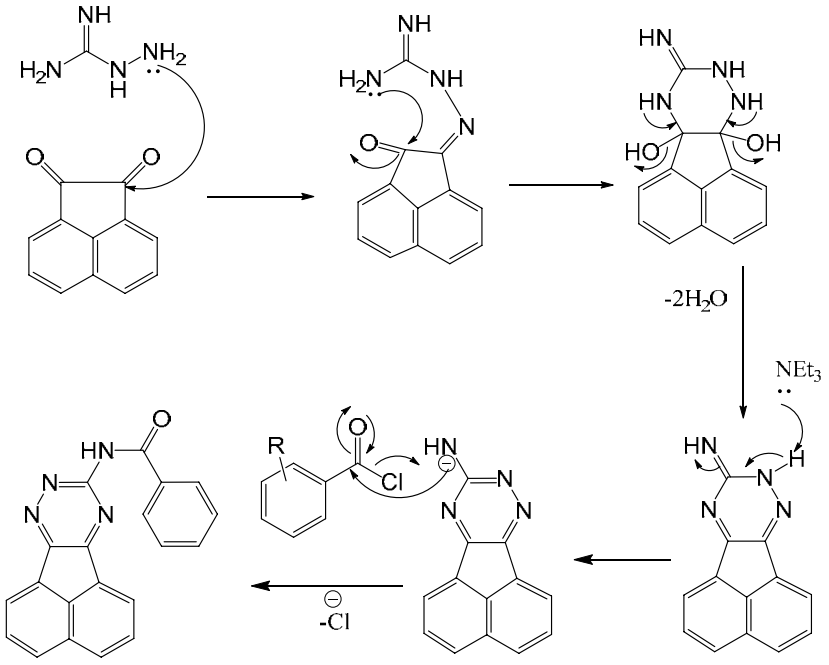



Scheme 3

*Table 1*

Synthesis of acenaphto triazin benzamide derivatives (7 a-g)

| Entry | Ar | Product | Yield (%) | m.p. (°C) |
|---|---|---|---|---|
| 1 | Benzoyl chlorid | **5a** | 96 | 233-234 |
| 2 | 2-Chloro-benzoyl chlorid | **5b** | 92 | 256-257 |
| 3 | 4-Chloro-benzoyl chlorid | **5c** | 97 | 260-262 |
| 4 | 2-boromo-benzoyl chlorid | **5d** | 93 | 249-250 |
| 5 | 4-nitro-benzoyl chlorid | **5e** | 96 | 232-234 |
| 6 | Benzy bromid | **5f** | 89 | 234-236 |
| 7 | 2-chloro-benzyl chloride | **5g** | 88 | 233-234 |




Scheme 4

*Table 2*

*In vitro* cytotoxicity of all the synthesized compounds against a panel of three standard cancer cell lines as well as a normal lung cell line

| Name | $IC_{50}$ (μM ± SD) | | | |
|---|---|---|---|---|
| | **MCF-7** | **SKOV3** | **A549** | **MRC-5** |
| **5a** | 124.13 ± 3.83 | 163.22 ± 2.14 | 114.39 ± 1.07 | > 200 |
| **5b** | 76.52 ± 2.14 | 91.26 ± 1.93 | 59.26 ± 3.27 | 94.33 ± 1.65 |
| **5c** | 38.97 ± 1.83 | 65.15 ± 2.72 | 32.17 ± 2.65 | 72.24 ± 1.08 |
| **5d** | > 200 | > 200 | 153.08 ± 4.12 | > 200 |
| **5e** | > 200 | > 200 | > 200 | > 200 |
| **5f** | 128.72 ± 2.92 | 141.54 ± 3.16 | 91.83 ± 2.19 | 125.66 ± 1.71 |
| **5g** | 89.13 ± 2.30 | 65.15 ± 1.72 | 61.52 ± 3.05 | 93.18 ± 2.31 |
| **5h** | > 200 | > 200 | > 200 | > 200 |
| **doxourobicin** | <1 | <1 | <1 | - |
| ***cis*-platin** | 9.33 ± 1.07 | 14.65 ± 0.52 | 13.19 ± 2.11 | 18.25 ± 1.32 |

Cytotoxic activity of all compounds was appraised by standard 3-(4,5-dimethylthiazol-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The cells were harvested and plated in 96-well microplates at a density of $1 \times 10^4$ cells per well in 180 μL complete culture media. After 24 h incubation, each cell was treated with five different concentrations of the compounds ranging from 1 to 200 μM. After 72h, media were replaced with 150 μL media containing 0.5 (mg/ml) of MTT solution. Then media containing MTT were discarded and 150 μL dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The solutions were incubated overnight. The absorbance in individual wells was determined at 570 nm using Bio-Rad microplate reader (Model 680). Data was calculated and expressed as the 50% inhibitory concentrations ($IC_{50}$), which were tested three times for each complex. Data are presented as mean ± SD.

Among the synthesized compounds, (N-(ace-naphtho[1,2-e][1,2,4]triazin-9-yl)-4-chlorobenzamid (5c) showed the highest cytotoxic effects on A549 and MCF-7 cells with $IC_{50}$ of 32.17 μM and 38.97 μM, respectively. However, 5c and 5g showed the best cytotoxicity against SKOV3 with $IC_{50}$ of 65.15 μM.

It seems that cytotoxic activities of triazine derivatives (5a-g) on the studied cell lines were dependent not only on the position of substituents on phenyl ring but also the nature of substitution on the phenyl ring. In this class, compounds 5d and 5e which possessing bromo and nitro substituent, showed lower cytotoxic activity compared to their chloro substituent analogues. It seems that compounds with chloro substitution on the para position, 5c, was also showed better cytotoxicity in compared to other substituent analogues in meta or ortho positions.

The effects of these synthesized triazine derivatives on the proliferation of MCR-5 (normal lung cell line), showed highest selectivity of the compounds between tumorigenic (A549) and non-tumorigenic (MRC-5) cell lines.

All prepared compounds were characterized with FT-IR, $^1$H NMR, $^{13}$C NMR spectroscopy. In the $^1$H NMR spectroscopy peak of the (NH) group can be seen in δ= 7.42 ppm. The simplicity of the reaction was more emphasized when the work-up of all the products carried out with simple crystallization and no need to other methods or technique, for purification of products.

## CONCLUSIONS

In summary, we established the simple synthesis pathway for the preparation of acenaphtho triazin benzamid derivatives through two step condensation reactions that started from the reaction of acenaphthoquinone and guanidine and then, the reaction continued to react with benzoyl chloride derivatives to form the final products in good yields. cytotoxic activity of prepared compounds showed that compound 5c have the best cytotoxic activity against the selected cancer cell lines with good selectivity among normal and cancer lung

cell lines. Simplicity of operation, easy work-up and purification procedures are several advantages of this protocol.

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