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# Tetramethylguanidiniumchlorosulfonate ionic liquid (TMG IL): An efficient reusable catalyst for the synthesis of tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-ones under solvent-free conditions and evaluation for their in vitro bioassay

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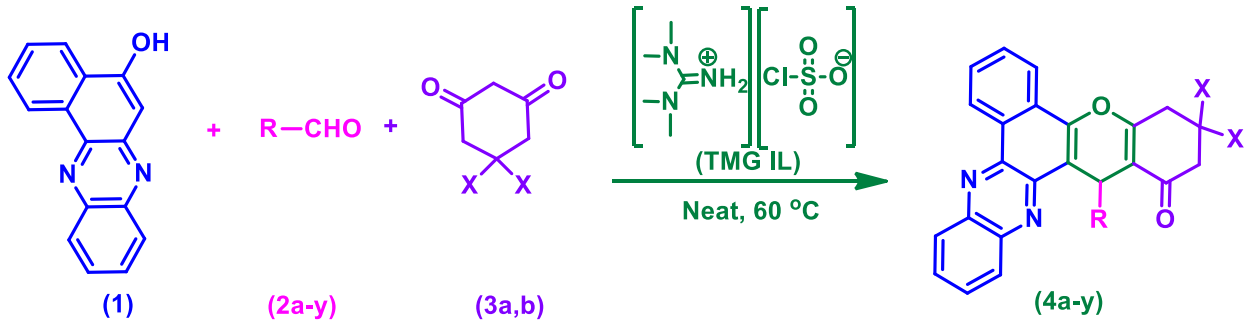
**Graphical abstract**




The scope and versatility of the green catalytic multi-component reaction has been demonstrated in this methodology are highly reactive, potent activity, ecologically cleaner route and reusability.

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**Abstract**

Medicinally important tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-ones are synthesized by straightforward, efficient and convenient approach of three-component reactions of aldehydes, benzo[a]phenazin-5-ol and active methylene compounds in the presence of tetramethylguanidiniumchlorosulfonate ionic liquid (TMG IL) under neat conditions. The TMG IL was used as solvent and as well catalyst under reusable conditions and observed higher product yields in shorter reaction time and environmentally benign reaction conditions are the merits of this reaction. The titled compounds were screened for their anti-oxidants and anti-cancer activities. Majority of them showed good bioassay activity.

**Keywords-** Tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-ones; anti-oxidants and anti-cancer activities; tetramethylguanidiniumchlorosulfonate ionic liquid (TMG IL).

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## 1. Introduction

The improvement and achievement of an efficient methodologies for the preparation of diversity oriented synthesis and intermediates of biologically relevant natural product like molecular frameworks is one of the main challenge for synthetic organic chemists.[1] Therefore, the researchers have made great efforts to find and develop new multi-component reactions (MCRs),[2] because which being competent and inexpensive methods and provide one of the most powerful platform to access diversity as well as manipulative simplicity, enhanced reaction rates, improved product selectivity, cleaner products and generation of molecular complexity in one-pot transformations.

Heterocycles and their derivatives have attracted significant interest owing to their comprehensive biological activities in the medicinal/ bioorganic/ pharmaceutical chemistry and as an important ligands in catalysis.[3] Amongst them, phenazinesis a nitrogen containing heterocyclic molecules which are well understood through its wide usage as a pharmacophore and synthon in heterocyclic chemistry.[4] Interestingly, a class of phenazines derivatives has been regularly studied because of its comparatively higher stability and concurrent monoimino character. Various chemotherapeutic agents possessing this class of phenazines moiety and have been studied for their potent antimicrobial, anti-inflammatory, antimalarial, trypanocidal, antiplateletantidepressant and antitumor activities.[4,5]

Now-a-days all over the world cancer is the most serious health problems. In this regards the researcher have been searching and finding an effective clinical approaches for the treatment of cancer and search for novel anti-cancer drugs from the past several decades. With wider applications and association of anti-cancer activities of natural and synthetic phenazines derivatives have been regularly searching in the literature since 1959.[4,6]

On the other hand, chromenes are also an important class of heterocyclic compounds which are shown attractive pharmacological properties such as antitumor, antivascular, antioxidant, antimicrobial, sex pheromone, TNF-α inhibitor, cancer therapy and central nervous system (CNS) activities.[7] Even though having attracted vast attention[4,8] on these two class of compounds incorporation of both, phenazine and tetrahydro-1*H*-benzo[*a*]chromene motifs have rarely been describe. In this connection recently reported a synthetic methodology for the synthesis of benzo[a]chromeno[2,3-c]phenazine derivatives,[9] even though, this methodology still has some more disadvantages such as long reaction time, homogenous catalysis and non-recyclable conditions. Further, a simple and better MCR was reported for synthesis of phenazines derivatives in water-etrhanol and common acid solvent medium at 80 °C. But used here the toxic $H_2SO_4$ and expensive phosphotungstic acid as catalyst.[10] Therefore we sought to develop a facile and versatile method for the combinatorial synthesis of tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones library for biological screening.

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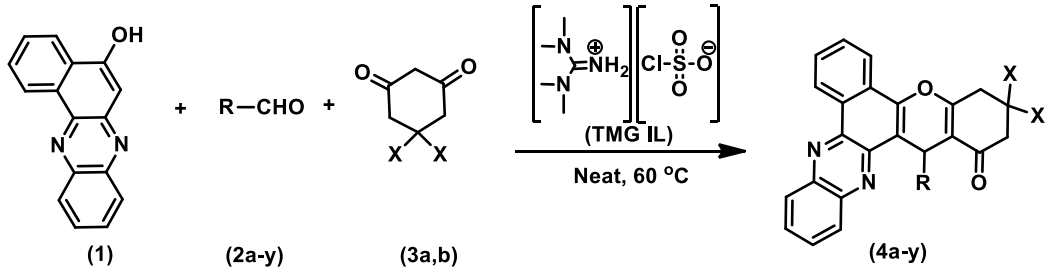
Recently, with greater selectivity even at higher temperature, ease of handling, environmental compatibility, reusability and ease of isolation of sulfonic acid functionalized ionic liquids have received great attention. Therefore, they have been widely used as solvent and catalysts in organic reactions.[11] In this connection, tetramethylguanidiniumchlorosulfonate ionic liquid (TMG IL) has find numerous advantages over conventional ionic liquids, such as ease of handling, less cost, non-corrosive, recyclable and thermally stable eco-friendly ionic liquid catalyst. Thus, it has been selected as an alternative solid heterogeneous catlyst for conventional ILs.

By collecting our previous knowledge on usage of ionic liquids as solvent and catalyst, and as a part of our continuing interest in the development of economic viability, greater selectivity, green and efficient new synthetic methods in the organic reactions[11j,12] we have report the synthesis of tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones (4a-y) by the condensation reactions of benzo[*a*]phenazin-5-ol (1) with various aldehydes (2a-y) and an active methylene compounds (3a,b) in the presence of catalytic amount (5 mol%) of TMG IL under solvent-free condition at 60 °C (Scheme 1).

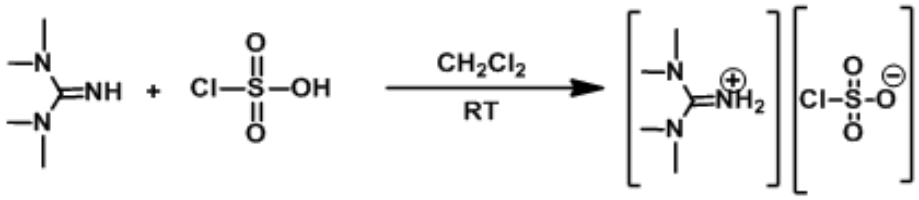



**Scheme 1.** Synthesis of tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones (**4a-y**).

## 2. Results and discussion

### 2.1. Chemistry

At first, due to the importance and development of an easy and efficient method for the preparation of TMG IL was reported by simple condensation reaction of tetramethylguanidinium (TMG) and chlorosulfonic acid in $CH_2Cl_2$ at room temperature (Scheme 2). The obtained white powdered, TMG IL, was characterized by elemental analysis, FT-IR and NMR spectroscopies.



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**Scheme 2.** Synthesis of TMG IL

The Fig. 1 showed the FT-IR spectrum of the TMG IL and two observed absorption bands at 3474 & 3398 indicating that the $NH_2$ stretching. The O=S=O asymmetric and symmetric stretching peaks observed at 1264 and 1174 $cm^{-1}$, respectively, and an S–O stretching peak at 720 $cm^{-1}$, which precisely confirmed the -$SO_3$ group linkage. Further, the C-N vibrations at 1630 $cm^{-1}$ indicated the presence of an imine.

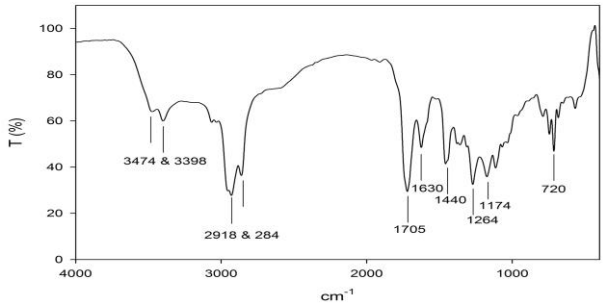


**Fig.1.** IR spectrum of TMG IL

The $^1H$ and $^{13}C$ NMR spectra of TMG IL were recorded in DMSO-$d_6$ and $D_2O$ (Fig. 2). $^1H$ NMR spectrum in DMSO-$d_6$ shown the methyl group protons at 2.87 ppm and $NH_2$ protons at 7.78 ppm (Fig. 2(i)). While carried out the spectrum in $D_2O$ the $NH_2$ protons are disappeared due to chemical exchange and observed a peak at 4.80 ppm by H-D (Fig. 2(ii)). On the other hand two carbon signals are observed in the $^{13}C$ NMR spectrum in both solvents at 161.0 & 160.7 ppm as singlet for C=N and at 39.4 & 39.8 as singlet signal for methyl group in DMSO-$d_6$ (Fig. 2(iii)) and $D_2O$ (Fig. 2(iv)) respectively. No more signals for $ClSO_3H$ are observed between 11 ppm and 14 ppm is indicating/ confirmation that the formation of the TMG IL.

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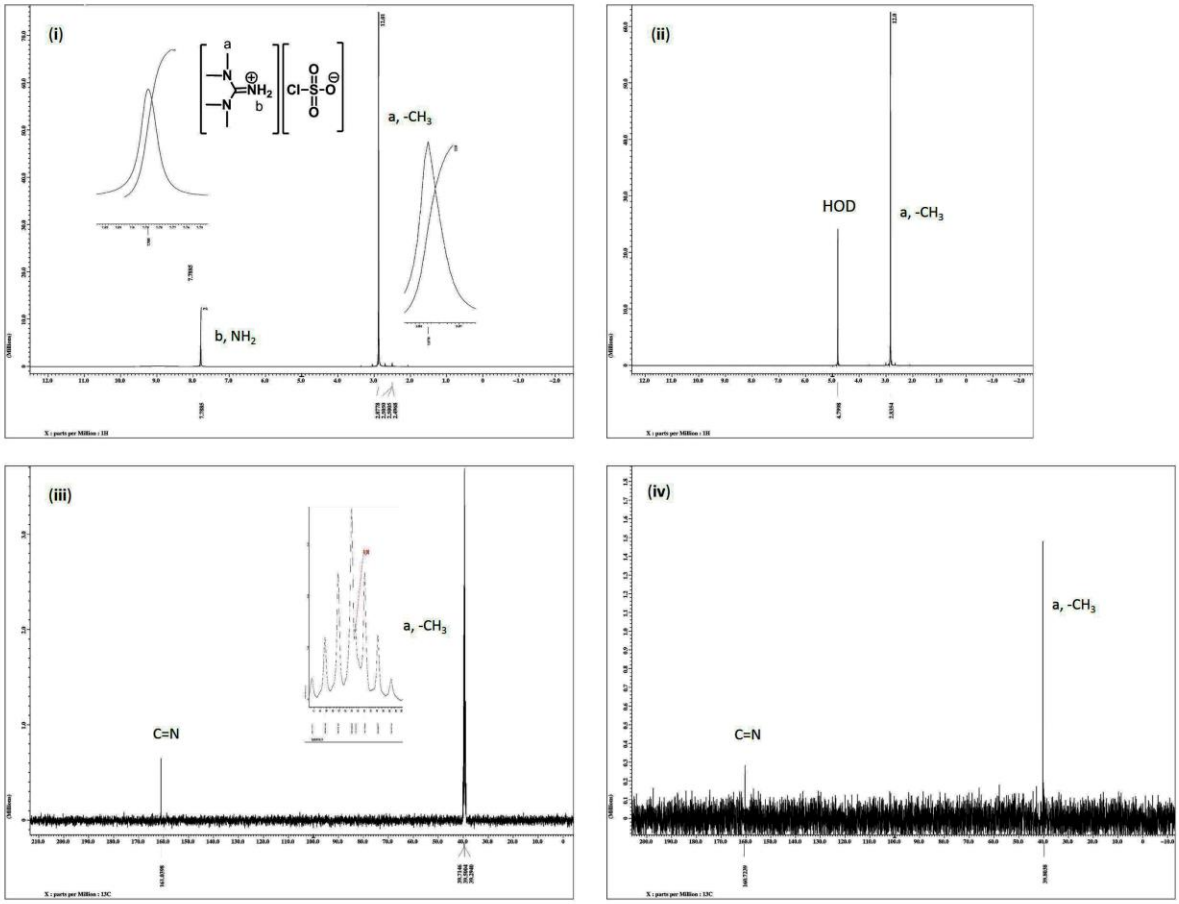


**Fig. 2.** NMR spectra of TMGL IL; (i) & (ii) are PMR spectra in DMSO-$d_6$& $D_2O$ respectively and (iii) &(iv) are CMR spectra in DMSO-$d_6$& $D_2O$ respectively.

Preliminary investigation of the title compounds was carried out on benzo[*a*]phenazin-5-ol (1, 1mmol), 4-ethoxybenzaldehyde (2a, 1mmol), and 5,5-dimethylcyclohexane-1,3-dione (3a, 1mmol) as a model reaction and TMG IL as catalyst to optimize the reaction conditions (Table 1). At the beigining, even the reaction was carriedout at rt-100 °C under neat conditions after prolonged reaction time could not resulted the product (Table 1, entries 1–4). Therefore, we investigated the reaction by using TMG IL (5 mol%) at 60 °C under solvent-free condition for a suitable catalytic condition in our search. In this context surprisingly we observed that the desired product in excellent yield within a much shorter reaction time (Table 1, entry 5) and without any side-product formation. To merit the catalytic activity of TMG IL a comparison study was conducted with supported ILs, such as, tri ethyl amine chlorosulfonate ionic liquid

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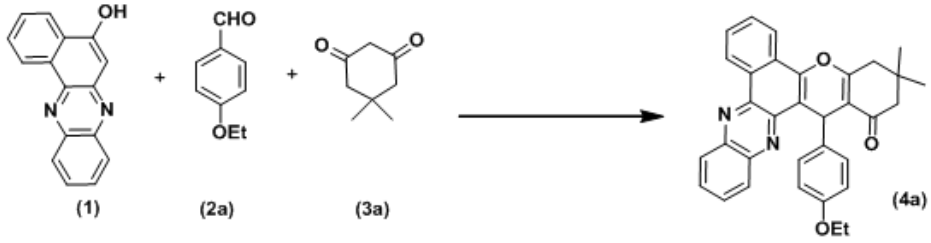
($Et_3N$ IL), di ethyl amine chlorosulfonate ionic liquid (DEA IL), di propyl amine chlorosulfonate ionic liquid (DPA IL) and di butyl amine chlorosulfonate ionic liquid (DBA IL) and also used its parental compounds, TMG and $ClSO_3H$ as catalysts under the similar reaction conditions and observed moderate to good catalytic properties (Table 1, entries 6-11). From these results concluded that the TMG IL is an efficient catalyst for the synthesis of tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones. In the rate of condensation reaction the substrate to catalyst ratio played an important role. Therefore, herein also studied the model reaction using 2 mol%, 5 mol% and 10 mol% quantities of TMG IL to obtaining maximal product yield, 82, 95 and 95%, respectively (Table 1, entries 5, 12 and 13). These results demonstrated that 5 mol% of TMG IL was sufficient fro the reaction.

After that we went with 5 mol% of TMG IL to check the effect of temperature and solvent on model reaction and observed lower rate reactions and poor yields (Table 1, entries 14–17). However, the solvent-free condition resulted excellent product yield. Therefore, 5 mol% of TMG IL catalyst under solvent-free conditions at 60 °C were determined as the optimal reaction conditions for the titled compounds synthesis (Table 1, entry 5).

**Table 1** Optimization of reaction conditions for the synthesis of 4a[a]




| Entry | Catalyst | Solvent | Tem. (°C) | Time (min) | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | Neat | Neat | RT | 450 | -- |
| 2 | Neat | Neat | 60 | 450 | 25 |
| 3 | Neat | Neat | 80 | 450 | 25 |
| 4 | Neat | Neat | 100 | 400 | 20 |
| 5 | TMGIL (5 mol%) | Neat | 60 | 45 | 95, 93, 90, 89,87 |
| 6 | TMG (5 mol%) | Neat | 60 | 182 | 55 |

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| 7 | $ClSO_3H$ (5 mol%) | Neat | 60 | 175 | 45 |
| 8 | $Et_3N$ IL (5 mol%) | Neat | 60 | 80 | 88 |
| 9 | DEA IL (5 mol%) | Neat | 60 | 83 | 85 |
| 10 | DPA IL ( 5 mol%) | Neat | 60 | 75 | 79 |
| 11 | DBA IL (5 mol%) | Neat | 60 | 76 | 86 |
| 12 | TMGIL (2 mol%) | Neat | 60 | 75 | 82 |
| 13 | TMGIL (10 mol%) | Neat | 60 | 45 | 95 |
| 14 | TMGIL (5 mol%) | Ethanol | 70 | 65 | 85 |
| 15 | TMGIL (5 mol%) | Acetonitrile | 65 | 80 | 75 |
| 16 | TMGIL (5 mol%) | THF | 60 | 82 | 78 |
| 17 | TMGIL (5 mol%) | Benzene | 80 | 90 | 70 |

[a]Reaction of benzo[*a*]phenazin-5-ol (1, 1mmol), 4-ethoxybenzaldehyde (2a, 1mmol), and 5,5-dimethylcyclohexane-1,3-dione (3a, 1mmol); [b]Isolated yield; [c]Catalyst was reused five times.

In the context of financial viability and sustainable development of catalyst the TMG IL was recovered by addition of ethyl acetate to the reaction mixture and separated by simple filtration. The precipitated TMG IL was washing twice with ethyl acetate (15+15 mL) and drying in vacuum and it used for five successive cycles, furnishing the corresponding 4a, with 95, 93, 90, 89 and 87% isolated yields (Table-1, entry 5) which confirmed the effectiveness of the catalyst for multiple usages (Fig.3). Therefore, as compared to the other reported methods in the cost analysis of reaction protocol makes it quite economical.

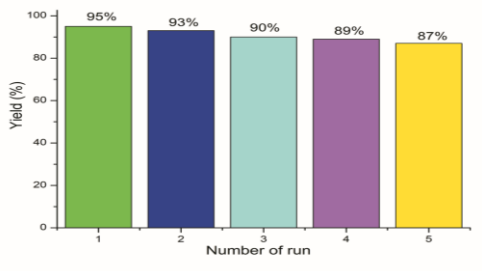


**Fig.3.** Reusability of the TMG IL

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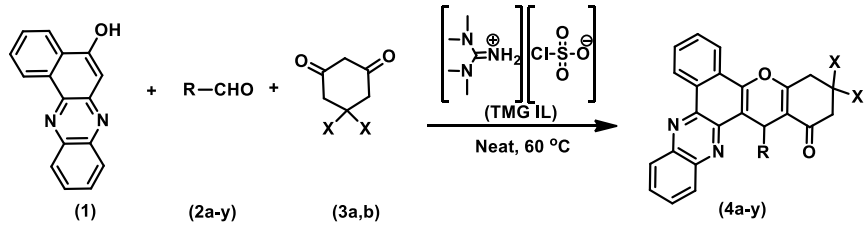
The obtained remarkable results on model reaction, encouraged us to show the generality and scope of this new protocol, applied the optimized conditions to synthesise a range of tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones using TMG IL (Table 2). As can be seen from Table 2, most of the reactions proceeded very efficiently and produced excellent product yields by simple filtration, without chromatography or cumbersome work-up procedure. The catalyst can be easily separated from the product as described above and reused without a significant loss in its catalytic activity. All the newly synthesised compound's structures were determined by $^{1}$H NMR, $^{13}$C NMR and HRMS.

**Table 2** Synthesis of tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-ones (4a-y)[a]




| Entry | Aldehyde R | X | Product | Time (min) | Yield[b] (%) | mp (°C) |
|---|---|---|---|---|---|---|
| 1 | 4-OEt-$C_6H_4$ | $CH_3$ | 4a | 45 | 95 | 223-225 |
| 2 | 2-5-Me-$C_6H_3$ | $CH_3$ | 4b | 56 | 90 | 286-288 |
| 3 | $C_8H_6O_3$ | $CH_3$ | 4c | 52 | 88 | 230-232 |
| 4 | 2-Me- $C_6H_4$ | $CH_3$ | 4d | 58 | 90 | 248-250 |
| 5 | 3-Br-$C_6H_4$ | $CH_3$ | 4e | 60 | 90 | 239-241 |
| 6 | 2-Br-$C_6H_4$ | $CH_3$ | 4f | 47 | 91 | 247-249 |
| 7 | 3-OH-$C_6H_4$ | $CH_3$ | 4g | 46 | 89 | 209-211 |
| 8 | 3-OMe-$C_6H_4$ | $CH_3$ | 4h | 51 | 91 | 222-224 |
| 9 | 4-CN-$C_6H_4$ | $CH_3$ | 4i | 50 | 89 | 236-238 |
| 10 | 4-F-$C_6H_4$ | $CH_3$ | 4j | 61 | 92 | 232-234 |
| 11 | 2-OMe-$C_6H_4$ | $CH_3$ | 4k | 64 | 93 | 280-282 |

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| 12 | $C_9H_7NO$ | $CH_3$ | 4l | 58 | 89 | 240-242 |
| 13 | 3-4-5-OMe-$C_6H_2$ | $CH_3$ | 4m | 53 | 90 | 239-241 |
| 14 | $C_5H_4OS$ | $CH_3$ | 4n | 59 | 90 | 259-261 |
| 15 | 3-$NO_2$-$C_6H_4$ | $CH_3$ | 4o | 57 | 91 | 280-282 |
| 16 | 3-F-$C_6H_4$ | $CH_3$ | 4p | 62 | 90 | 313-315 |
| 17 | 4-$NO_2$-$C_6H_4$ | $CH_3$ | 4q | 60 | 90 | 296-298 |
| 18 | 4-N-Di-Me- $C_6H_4$ | $CH_3$ | 4r | 64 | 93 | 248-250 |
| 19 | 4-OEt-$C_6H_4$ | H | 4s | 66 | 90 | 224-226 |
| 20 | 4-Iso-Pro-$C_6H_4$ | H | 4t | 60 | 95 | 250-252 |
| 21 | 2-Cl-$C_6H_4$ | H | 4u | 56 | 90 | 230-232 |
| 22 | 2-OMe-$C_6H_4$ | H | 4v | 65 | 91 | 302-304 |
| 23 | 3-OMe-$C_6H_4$ | H | 4w | 61 | 95 | 241-243 |
| 24 | 3-Cl-$C_6H_4$ | H | 4x | 64 | 90 | 289-291 |
| 25 | 3-F-$C_6H_4$ | H | 4y | 52 | 92 | 285-287 |

[a]Reaction of benzo[*a*]phenazin-5-ol (1, 1mmol), aldehydes (2a-y, 1mmol), and active methylene compounds (3a, b, 1mmol); [b]Isolated yield.

The TMG IL catalyzed synthetic sequences of titled compounds are presented schematically in Scheme 3 and it may proceed via the ortho-quinonemethides (o-QM) intermediate. At beginning takes the nucleophilic addition of benzo[a]phenazin-5-ol (1) to aldehydes (2a-y) and subsequent Michael addition of the o-QM with enolic form of cyclic 1,3-dicarbonyl (3a,3b) followed by addition of the benzyl hydroxy moiety to the carbonyl of ketone (6) provides cyclic hemiketal (7) which on dehydration afforded 4a-y.

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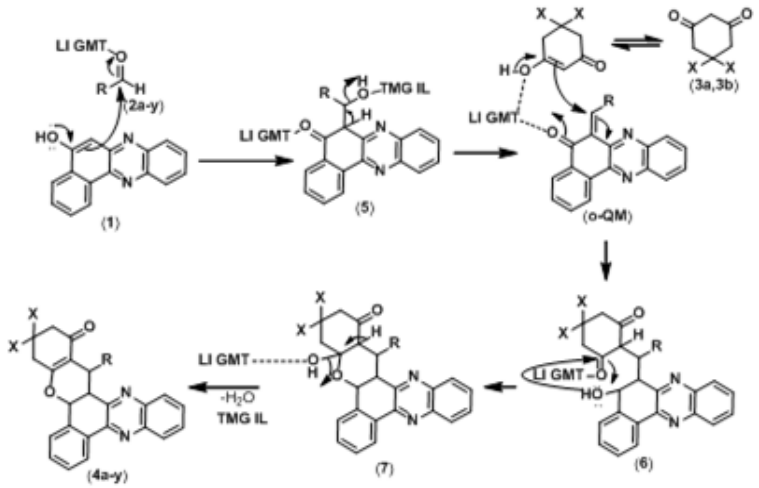


**Scheme 3.** Schematic presentation of synthesis of tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones (4a-y)

### 2.2. Pharmacological screening

An increased interest has been developed to use anti-oxidants in recent years for the medical purposes. Thus, in the preventing and/or treatment of diseases drugs are considered, that are having anti-oxidant and free radical scavenging properties. Therefore, herein, we screened the anti-oxidant activity and followed by the cytotoxic activity of all the synthesized compounds, since, the anti-oxidants help to prevent cancer and other cardiovascular diseases.

#### 2.2.1. Anti-oxidant activity

The free radicals which are generated in many bioorganic redox-processes of the body may damage in various components by inducing oxidative process and it has been implicated in a number of life-limiting chronic diseases and aging. These free radicals with high reactivity in the body can be neutralized with an anti-oxidant by donating an electron or hydrogen radical and thus free radical scavengers (Scheme 4). For the newly synthesized titled compounds explored their free radical scavenging ability in three type of *in vitro* assay experiments, such as, 1,1-diphenyl-2-picrylhydrazyl (DPPH),[13] hydroxyl ($H_2O_2$)[14] and reducing power (RP).[15] Herein, we used the Vitamin-C as standard anti-oxidant to measure the *in vitro* inhibitory concentration of 50% ($IC_{50}$) (Eq.-1). Most of the titled compounds shown good anti-oxidant activities in represented methods (Table 3).

$$\% \text{ of scavenged} = \frac{(A_{cont.} - A_{test})}{A_{cont.}} \times 100 \quad \text{(Eq.-1)}$$

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Where, $A_{cont}$. is the absorbance of the control (containing all reagents except the test compound, blank sample) and A test is the absorbance of the test compound.

**2.2.2. DPPH scavenging activity**

Nitrogen centered stable DPPH free radical is one of the widely accepted and often used tool for estimating free radical scavenging activity of anti-oxidants because it is a simple, rapid, and inexpensive method. In radical scavenging activity determination of anti-oxidants DPPH (purple in colour) can accept an electron or hydrogen radical from anti-oxidant and forms a non-radical DPPH (de-colorization) in stoichiometry (Scheme 4) and has been used to measure anti-oxidant properties by a change in the absorbance produced in this change.

Herein, most of the titled compounds showed good to excellent scavenging activity (Table 3) with good radical and/or hydrogen donating capacity (Scheme 4). In them most of the titled compounds,4c, 4k, 4l, 4v, 4n, 4x, 4m, 4w, 4f,and 4r showed excellent scavenged activity, 88.8, 87.3, 87.0, 86.9, 86.6, 85.4, 85.3, 85.1, 81.1 and 80.6 respectively, when compare with the positive control standard, Vitamin-C (91.2%). The remaining compounds are also exhibited good radical scavenging activity (Fig. 4).

**2.2.3. Hydroxyl radical ($H_2O_2$) scavenging activity**

Hydroxyl radicalisone of the most reactive oxygen species (ROS) that attacks almost every molecule in the body. Most of the newly synthesized titled compounds showed good to excellent hydroxy radical scavenging activity (Table 3). Compounds,4c, 4k, 4l, 4v, 4m, 4m, and 4w were showed excellent OH radical scavenging activity of 88.8, 87.2, 87.0, 86.9, 85.3 and 85.1% respectively when compare with others. The remaining compounds also exhibited good radical scavenging activity when compare to the positive control ascorbic acid (92.1%) (Fig. 4).

**2.2.4. Reducing power (RP) scavenging activity**

The compound potential anti-oxidant activity may indicate by its significant reducing power capability. With increasing nature of ready availability of either non-bonding electrons or labile hydrogen of compounds the reducing power also increased (Table 3). The results showed (Fig. 4) that the compounds, 4k, 4m, 4n, 4c, and 4w are exhibited more scavenging activity of 94.4, 93.2, 92.0, 91.2and 90.4% respectively. The remaining most of the compounds also showed good scavenging activity when compare with the positive control ascorbic acid (95.6%).

With the above radical scavenging activities indicated that the titled compounds can be considered as promising anti-oxidant agents in drug chemistry.

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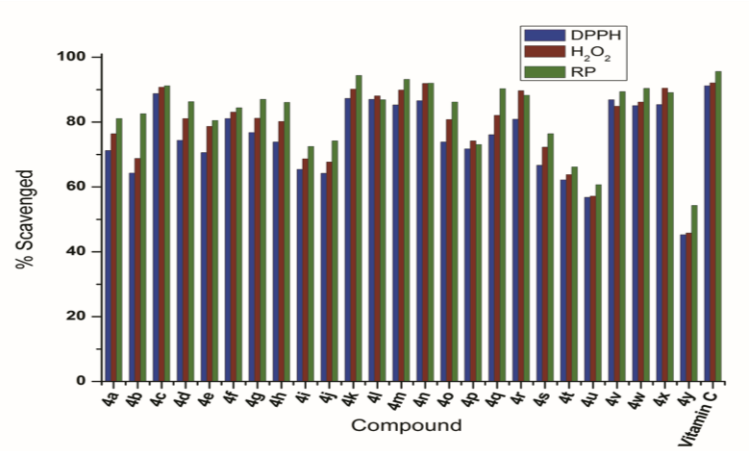


**Fig. 4.** Anti-oxidant activity of the titled compounds in three methods: All the radical scavenging activities represent with the standard Vitamin-C and the data expressed as mean ± S.D. (n = 3).

**Table 3** Anti-oxidant activity of titled compounds (4a-y)

| Compound | Inhibition % | | |
|---|---|---|---|
| | DPPH | $H_2O_2$ | RP |
| 4a | 71.27 | 76.4 | 81.1 |
| 4b | 64.3 | 68.8 | 82.6 |
| 4c | 88.8 | 90.8 | 91.2 |
| 4d | 74.4 | 81.1 | 86.3 |
| 4e | 70.6 | 78.7 | 80.5 |
| 4f | 81.1 | 83.1 | 84.4 |
| 4g | 76.8 | 81.2 | 87.0 |
| 4h | 73.9 | 80.2 | 86.1 |
| 4i | 65.4 | 68.7 | 72.5 |
| 4j | 64.2 | 67.7 | 74.2 |

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|---|---|---|---|
| 4k | 87.3 | 90.2 | 94.4 |
| 4l | 87.0 | 88.1 | 86.9 |
| 4m | 85.3 | 89.9 | 93.2 |
| 4n | 86.6 | 91.9 | 92.0 |
| 4o | 73.9 | 80.8 | 86.2 |
| 4p | 71.8 | 74.2 | 73.1 |
| 4q | 76.1 | 82.1 | 90.3 |
| 4r | 80.9 | 89.7 | 88.3 |
| 4s | 66.7 | 72.3 | 76.4 |
| 4t | 62.2 | 63.8 | 66.2 |
| 4u | 56.8 | 57.2 | 60.7 |
| 4v | 86.9 | 84.9 | 89.4 |
| 4w | 85.1 | 86.2 | 90.4 |
| 4x | 85.4 | 90.5 | 89.1 |
| 4y | 45.3 | 45.8 | 54.3 |
| Vitamin - C | 91.2 | 92.1 | 95.6 |

**2.2.5. Anticancer activity**

Now-a-days frequently reported the anti-cancer activity of many heterocyclic compounds because of them are auxiliary drugs co-administers with various formulation againstvarious anti-cancer cell lines. Therefore, our attention was turned to estimate their anti-cancer activity once the titled compounds shown excellent anti-oxidant activities.

All the titled compounds were assessed *in vitro* anti-proliferative activity against HeLa (human cervical cancer) and SK-BR-3 (human breast adenocarcinoma) cell lines. After 24 h of treatment (MTT assay) the numbers of live cells were measured and determined their cytotoxic activity[16] with half maximal inhibitory concentrations ($IC_{50}$) (Table 4).

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The results demonstrated that,compared with that of standard anticancer drugs, Etoposide and Camptothecin compounds 4c, 4l, 4k, 4v, 4n, 4x, 4mand 4u possessed closer anti-proliferative activity. Among them, compounds 4c, 4l and 4k has the highest activity,3.28±0.61, 4.01±0.62 and 4.31±0.71 µg/ mL for HeLa and 2.24±0.51, 3.12±0.59 and 3.81±0.72 µg/mL for SK-BR-3 cells respectively. The remaining compounds showed almost closer level of activity.

In the present *in vitro* investigation used healthy/ normal human breast cells (HBL 100) to test the effect of toxic nature of titled compounds and they did not shown significant effect at lower concentrations. However, with increasing concentration (100 µg/mL in 24 h) of titled compounds produced significant toxicity against the normal cells.

**Table 4** Anti-proliferativeactivity* of test compounds (4a-y) on HeLa and SK-BR-3 Celllines[a]

| Compound | ($IC_{50}$ in µg/mL)[§] | |
|---|---|---|
| | HeLa | SK-BR-3 |
| 4a | 51.26±0.30 | 57.53±0.84 |
| 4b | 55.21±0.15 | 62.41±0.53 |
| 4c | 3.28±0.61 | 2.24±0.51 |
| 4d | 32.21±0.46 | 39.50±0.91 |
| 4e | 40.94±0.15 | 45.23±0.39 |
| 4f | 25.49±0.21 | 25.28±0.29 |
| 4g | 77.38±0.25 | 81.91±0.87 |
| 4h | 82.63±0.26 | 87.92±0.56 |
| 4i | 29.18±0.42 | 25.58±0.53 |
| 4j | 46.15±0.59 | 51.26±0.59 |
| 4k | 4.31±0.71 | 3.81±0.72 |
| 4l | 4.01±0.62 | 3.12±0.59 |
| 4m | 8.18±0.27 | 12.57±0.75 |
| 4n | 5.55±0.72 | 5.15±0.39 |
| 4o | 69.49±0.78 | 78.89±0.60 |
| 4p | 74.56±0.98 | 82.25±0.81 |
| 4q | 31.87±0.93 | 49.07±0.75 |

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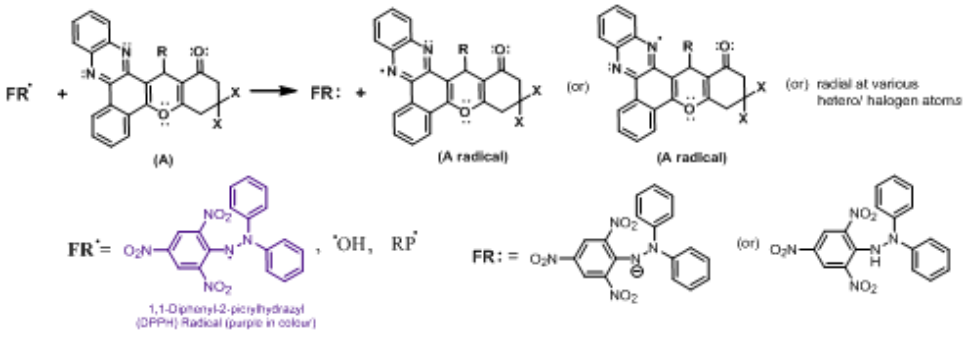
|---|---|---|
| 4r | 25.96±0.56 | 43.71±0.31 |
| 4s | 18.78±0.97 | 30.83±0.87 |
| 4t | 11.78±0.86 | 26.60±0.71 |
| 4u | 8.59±0.97 | 15.21±0.76 |
| 4v | 5.13±0.42 | 4.48±0.27 |
| 4w | 79.28±0.96 | 84.95±0.82 |
| 4x | 7.93±0.56 | 10.64±0.57 |
| 4y | 12.13±0.89 | 24. 82±0.82 |
| Etoposide | 9.23±0.49 | 4.92±0.40 |
| Camptothecin | 1.65±0.32 | 1.28±0.19 |

*With different concentrations of test compoundswere treated for 24 h on exponentially growing cells and cell growth inhibition was analyzed by MTT assay; [a]to calculate the linear regression equation used the mean percentage decrease in cell number of five independent experiments; [§]50% concentration decrease ($IC_{50}$) of cell number in the absence of an inhibitor as compared with that of the control cultures. The values are five individual observations inmean±SE (SE = standard error).

**2.2.6. Structure activity relationship (SAR)**

The titled heterocyclic compounds were shown potent bioassay nature in both anti-oxidant and anti-cancer activities may due to the presence of the basic core unit structure (A) with combination of non-bonded electron pairs on either heteroatom or halogens and therefore, can interact easily with ROS/cancerous cell lines.




**Scheme 4.** Potent bioassay nature of titled compounds (4a-y) by radical scavenging activity

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The other factors, such as substituents, on the core unit have not shown much more deviation on bioassay of titled compounds. It's once again proved that the 1,2-dihydrophenazine unit has great bioassay in biological systems. In the anti-oxidant bioassay the compounds,which are possess with good electron donor substituents on the basic core structure (A), could affecting the ability of titled compounds to interact with the peripheral and thereby influencing the scavenging activity on ROS. On the other hand, the titled compounds are also shown good anti-proliferative activity on the PANC-1 cell line. The compounds with labile hydrogen on nitrogen, 4c and 4l are shows highest inhibitory bioassay. Good anti-cancer activity was also observed for the other compounds without much deviation based on the substituents on basic core unit, A.

## 3. Conclusion

We have reported a series of bioactive tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones in a simple, green, and efficient multi-component one-pot synthesis from readily available starting materials and tetramethylguanidiniumchlorosulfonate ionic liquid as an efficient catalyst under solvent-free conditions. This protocol demonstrated that the tetramethylguanidiniumchlorosulfonate ionic liquid is an alternative catalyst in green chemistry for those known strategies in multi-component reactions. Because it's tolerance towards a wide range of reactant and preoducing reasonable product yields. The tilled compounds were screened for their *in vitro* anti-oxidant and found to be most of the compounds are effective against reactive oxygen species. Majority of them also have excellent *in vitro* anti-cancer activity on two human cancer cell lines, HeLa and SK-BR-3, compare with that of standard drugs.

## 4. Experimental

### 4.1 Material and methods

Chemicals were purchased from Aldrich and Alfa Aesar Chemical Companies. NMR spectra were recorded in ppm in $CDCl_3$ on a Jeol JNM ECP 400 NMR instrument using TMS as an internal standard. Mass spectra were recorded on a Jeol JMS-700 mass spectrometer. All melting points were determined using open capillaries on an Electrothermal-9100 (Japan) instrument.

### 4.2 Synthesis of TMG IL

To a stirred and cold solution (0 °C) of chlorosulfonic acid (2 mmol) in $CH_2Cl_2$ (10 mL) in a 50 mL reaction flask was added the TMG solution (2 mmol) in $CH_2Cl_2$ (15 mL) over a period of 5 min (Scheme 2). After completion of the addition, continue the stirrng for further 40 min at room temperature and then concentrated it by rotary evaporation. To afford TMG IL as a white solid

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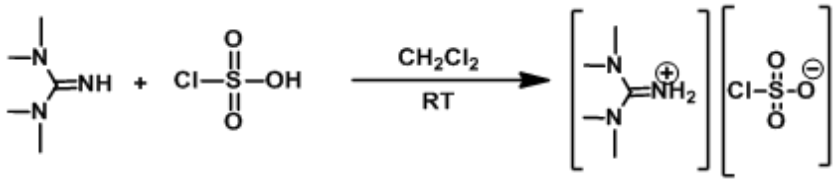
the obtained residue was washed with $CH_2Cl_2$ twice (15+15 mL) and dried at 70 °C by rotary evaporation without using any further specific purification.




**Scheme 2.** Preparation of TMG IL

TMGIL: white solid, mp 41–43 °C; IR (KBr): ν ($cm^{-1}$) 3474, 3398, 1630, 1264, 1174, 884, 720, 655, and 595; $^1$H NMR (400 MHz, $D_2O$): δ (ppm) 2.83 (s, 12H, \$CH_3$ × 4); $^{13}$C NMR (100 MHz, $D_2O$): δ (ppm) 160.7, 39.8; $^1$H NMR (400 MHz, DMSO-$d_6$): δ (ppm) 7.78 (s, 2H, \$NH_2$) 2.87 (s, 12H, \$CH_3$ × 4); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ (ppm) 161.0, 39.4. Anal. Cacld for $C_5H_{14}N_3O_3SCl$: C, 25.92; H, 6.09; N, 18.14; S, 13.84. Found: C, 26.96; H, 6.01; N, 19.57; S, 13.55.

**4.3 Synthesis of 16-(4-ethoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4a)**.

In a100 mL RB flask stirred the mixture of benzo[a]phenazin-5-ol (1, 1mmol), 4-ethoxybenzaldehyde (2a, 1mmol), and 5,5-dimethylcyclohexane-1,3-dione (3a, 1mmol) with TMG IL (5% mol) at 60 °C for 45 min. Finally the product (completion of reaction by TLC) was filtrated with 20 mL of ethyl acetate (EA) and the filtrate was denser under reduced pressure.The obtained solid product was washed with hexane and recrystallized from ethanol to afford the pure product. The precipitated TMG IL was washed with EA twice and then dried under vacuum before reuse.

**4.3.1. 16-(4-ethoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4a).** Yield 95%; yellow powder; mp 223-225 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.26 (dd, *J* = 2.0, 8.1 Hz, 1H), 8.29 (dd, *J* = 2.1, 8.0 Hz, 1H), 8.21-8.17 (m, 2H), 7.79-7.70 (m, 4H), 7.48 (d, *J* = 8.0 Hz, 2H), 6.67 (d, *J* = 8.0 Hz, 2H), 6.04 (s, 1H), 3.87-3.82 (q, 2H), 2.78 and 2.73 (AB System, *J* = 16.4 Hz, 2H), 2.39 and 2.32 (AB System, *J* = 16.4 Hz, 2H), 1.25 (t, *J* = 6.9 Hz, 3H), 1.19 (s, 3H), 1.14 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 163.4, 157.4, 147.0, 142.7, 142.1, 140.5, 140.4, 137.2, 130.5, 130.2, 130.0, 129.8, 129.5, 129.1, 128.5, 126.8, 125.7, 122.1, 116.6, 115.4, 113.9, 63.2, 51.1, 41.4, 32.8, 32.6, 29.6, 27.5, 14.9 ppm. HRMS (ESI, m/z): calcd for $C_{33}H_{28}N_2O_3$ ($M+H^+$) 500.210; found: 500.202.

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**4.3.2. 16-(2,5-dimethylphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4b).** Yield 90%; yellow powder; mp 286-288 °C. $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 9.21 (d, *J* = 8.0 Hz, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.14-8.11 (m, 2H), 7.80-7.69 (m, 4H), 6.695 (d, *J* = 6.9 Hz, 1H), 6.78 (s, 1H), 6.68 (d, *J* = 6.9 Hz, 1H), 5.88 (s, 1H), 3.24 (s, 3H), 2.74 (s, 2H), 2.38 and 2.28 (AB System, *J* = 16.4 Hz, 2H), 1.99 (s, 3H), 1.20 (s, 3H), 1.06 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.1, 163.1, 147.0, 144.0, 142.7, 142.1, 141.1, 140.7, 134.8, 134.6, 130.8, 130.0, 129.9, 129.8, 129.5, 129.3, 128.3, 127.1, 126.7, 125.5, 122.0, 117.8, 116.4, 51.2, 41.5, 32.6, 30.2, 29.6, 27.3, 21.3, 20.3 ppm. HRMS (ESI, m/z): calcd for $C_{33}H_{28}N_2O_2$ ($M+H^+$) 484.215; found: 484.211.

**4.3.3. 16-(benzo[d][1,3]dioxol-5-yl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4c)**. Yield 88%; yellow powder; mp 230-232 °C. $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 9.34-9.32 (m, 1H), 8.36-8.34 (m, 1H), 8.26-8.23 (m, 2H), 7.83-7.73 (m, 4H), 7.14 (d, *J* = 6.9 Hz, 1H), 7.04-7.02 (m, 1H), 6.69 (d, *J* = 6.9 Hz, 1H), 6.05 (s, 1H), 5.78 (dd, *J* = 1.4, 10.6 Hz, 2H), 2.82 and 2.74 (AB System, *J* = 16.4 Hz, 2H), 2.40 and 2.34 (AB System, *J* = 16.4 Hz, 2H), 1.20 (s, 3H), 1.14 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.1, 163.6, 147.2, 146.9, 145.9, 142.7, 142.0, 141.0, 140.9, 139.2, 131.0, 130.0, 129.6, 129.5, 128.5, 126.5, 125.6, 122.2, 122.0, 116.4, 115.3, 110.0, 107.8, 100.8, 51.1, 41.4, 33.3, 32.6, 29.6, 27.7 ppm. HRMS (ESI, m/z): calcd for $C_{32}H_{24}N_2O_4$ ($M+H^+$) 500.174; found: 500.170.

**4.3.4. 3,3-dimethyl-16-(o-tolyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4d).** Yield 90%; yellow powder; mp 248-250 °C. $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 9.30 (dd, *J* = 2.0, 8.0 Hz, 1H), 8.35 (dd, *J* = 2.2, 7.6 Hz, 1H), 8.24-8.20 (m, 2H), 7.83-7.73 (m, 4H), 7.43 (s, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.03 (t, *J* = 6.9 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.09 (s, 1H), 2.82 and 2.75 (AB System, *J* = 16.4 Hz, 2H), 2.39 and 2.33 (AB System, *J* = 16.4 Hz, 2H), 2.22 (s, 3H), 1.19 (s, 3H), 1.13 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.1, 163.1, 147.0, 144.3, 142.6, 142.0, 141.0, 137.6, 130.8, 130.1, 129.9, 129.8, 129.5, 129.4, 128.5, 128.3, 126.6, 126.2, 125.6, 122.0, 117.8, 116.3, 51.1, 41.4, 32.5, 30.1, 29.6, 27.3, 20.7 ppm. HRMS (ESI, m/z): calcd for $C_{32}H_{26}N_2O_2$ ($M+H^+$) 470.199; found: 470.192.

**4.3.5. 16-(3-bromophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4e).** Yield 90%; yellow powder; mp 239-241 °C. $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 9.32-9.30 (m, 1H), 8.39-8.33 (m, 2H), 8.24-8.21 (m, 1H), 7.83-7.76 (m, 4H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.30 (s, 1H), 7.06-7.01 (m, 1H), 6.86-6.82 (m, 1H), 6.22 (s, 1H), 2.77 (s, 2H), 2.40 and 2.34 (AB System, *J* = 16.4 Hz, 2H), 1.20 (s, 3H), 1.10 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 163.4, 148.0, 142.9, 142.1, 140.5, 140.3, 133.3, 130.2, 130.0, 129.9, 129.7,

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129.1, 128.7, 127.7, 126.9, 126.6, 125.7, 122.2, 115.8, 51.1, 41.6, 35.1, 32.4, 29.5, 27.6 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{23}BrN_2O_2$ ($M+H^+$) 535.431; found: 535.425.

**4.3.6. 16-(2-bromophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4f)**. Yield 91%; yellow powder; mp 247-249 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.31 (dd, *J* = 1.8, 7.2 Hz, 1H), 8.38-8.33 (m, 1H), 8.25-8.19 (m, 2H), 7.83-7.77 (m, 4H), 7.72-7.71 (m, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.15-7.13 (m, 1H), 7.05-6.99 (m, 1H), 6.05 (s, 1H), 2.83 and 2.76 (AB System, *J* = 16.4 Hz, 2H), 2.40 and 2.34 (AB System, *J* = 16.4 Hz, 2H), 1.20 (s, 3H), 1.14 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 196.8, 163.9, 147.3, 147.0, 142.6, 141.7, 141.0, 140.9, 132.3, 131.3, 131.1, 130.1, 130.4, 129.7, 129.5, 128.6, 128.1, 126.4, 125.6, 122.1, 115.6, 114.6, 51.0, 41.4, 33.7, 32.6, 29.6, 27.6 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{23}BrN_2O_2$ ($M+H^+$) 534.094; found: 534.098.

**4.3.7. 16-(3-hydroxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4g).** Yield 89%; yellow powder; mp 209-211 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.24 (dd, *J* = 2.1, 8.1 Hz, 1H), 8.30 (dd, *J* = 2.0, 8.6 Hz, 1H), 8.17-8.14 (m, 2H), 7.84-7.69 (m, 4H), 7.07-7.02 (m, 2H), 6.88-6.83 (m, 2H), 5.97 (s, 1H), 2.75 (s, 2H), 2.38 and 2.28 (AB System, *J* = 16.2 Hz, 2H), 1.19 (s, 3H), 1.05 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.1, 164.1, 152.7, 146.9, 142.5, 142.0, 141.5, 140.4, 130.9, 130.1, 129.7, 129.6, 129.1, 128.5, 126.6, 125.6, 122.0, 116.4, 114.9, 106.5, 51.0, 41.4, 33.7, 32.5, 29.9, 27.3 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{24}N_2O_3$ ($M+H^+$) 472.179; found: 472.172.

**4.3.8. 16-(3-methoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4h).** Yield 91%; yellow powder; mp 222-224 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.33 (dd, *J* = 2.1, 8.1 Hz, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.22-8.18 (m, 2H), 7.89-7.71 (m, 5H), 7.04-7.00 (m, 1H), 6.91-6.85 (m, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 6.19 (s, 1H), 3.69 (s, 3H), 2.77 and 2.72 (AB System, *J* = 16.4 Hz, 2H), 2.37 and 2.28 (AB System, *J* = 16.2 Hz, 2H), 1.19 (s, 3H), 1.08 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 196.7, 163.8, 159.3, 146.6, 140.8, 130.0, 129.7, 129.5, 128.8, 128.5, 126.6, 125.8, 122.1, 121.5, 115.3, 111.8, 52.2, 51.1, 41.4, 33.7, 32.6, 29.6, 27.6 ppm. HRMS (ESI, m/z): calcd for $C_{32}H_{26}N_2O_3$ ($M+H^+$) 486.194; found: 486.190.

**4.3.9. 4-(3,3-dimethyl-1-oxo-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-16-yl)benzonitrile (4i).** Yield 89%; yellow powder; mp 236-238 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.31 (d, *J* = 5.8 Hz, 1H), 8.34-8.14 (m, 3H), 7.82-7.79 (m, 4H), 7.79 (d, *J* = 6.9 Hz, 2H), 7.44 (d, *J* = 6.9 Hz, 2H), 6.08 (s, 1H), 2.83 (s, 2H), 2.41 and 2.32 (AB System, *J* = 16.4 Hz, 2H), 1.21 (s, 3H), 1.09 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 164.0, 150.6, 147.2, 141.5,

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140.9, 131.8, 130.3, 130.2, 130.0, 129.5, 129.2, 128.8, 125.6, 122.1, 114.8, 114.0, 110.1, 50.9, 41.3, 34.3, 32.6, 29.6, 27.4 ppm. HRMS (ESI, m/z): calcd for $C_{32}H_{23}N_3O_2$ ($M+H^+$) 481.179; found: 481.173.

**4.3.10. 16-(4-fluorophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4j).** Yield 92%; yellow powder; mp 232-234 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.41 (dd, *J* = 2.0, 8.1 Hz, 1H), 8.40-8.36 (m, 2H), 8.27-8.23 (m, 1H), 7.90-7.82 (m, 4H), 7.56-7.52 (m, 2H), 6.85-6.81 (m, 2H), 6.10 (s, 1H), 2.82 and 2.77 (AB System, *J* = 16.4 Hz, 2H), 2.43 and 2.37 (AB System, *J* = 16.4 Hz, 2H), 1.21 (s, 3H), 1.12 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 163.6, 162.4 (d, *J* = 241.2 Hz), 147.3, 142.7, 142.0, 139.7, 130.6, 130.5, 129.4, 128.9, 128.7, 126.8, 126.4, 126.0, 122.3, 116.0, 114.9, 114.7, 51.0, 41.4, 33.1, 32.6, 29.6, 27.5 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{23}FN_2O_2$ ($M+H^+$) 474.174; found: 474.168.

**4.3.11. 16-(2-methoxyphenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4k).** Yield 93%; yellow powder; mp 280-282 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.37 (d, *J* = 7.3 Hz, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 8.27 (t, *J* = 7.2 Hz, 2H), 7.85-7.81 (m, 5H), 7.16 (d, *J* = 6.9 Hz, 1H), 7.06 (t, *J* = 6.9 Hz, 1H), 6.56 (d, *J* = 7.3 Hz, 1H), 6.14 (s, 1H), 3.73 (s, 3H), 2.83 and 2.76 (AB System, *J* = 16.4 Hz, 2H), 2.37 (d, *J* = 16.2 Hz, 2H), 1.20 (s, 3H), 1.15 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.1, 164.2, 158.1, 147.5, 142.7, 142.6, 140.8, 133.2, 131.6, 130.9, 129.8, 129.7, 129.5, 129.4, 129.2, 128.1, 127.8, 126.7, 125.5, 121.9, 120.1, 114.7, 113.0, 110.9, 55.4, 51.1, 41.5, 32.5, 31.7, 29.8, 27.0 ppm. HRMS (ESI, m/z): calcd for $C_{32}H_{26}N_2O_3$ ($M+H^+$) 486.194; found: 486.188.

**4.3.12. 16-(1H-indol-3-yl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4l).** Yield 89%; yellow powder; mp 240-242 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.32 (d, *J* = 8.0 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.25-8.22 (m, 1H), 8.16-8.13 (m, 1H), 7.87-7.78 (m, 3H), 7.74-7.71 (m, 3H), 7.41 (d, *J* = 6.9 Hz, 2H), 7.09 (d, *J* = 6.9 Hz, 2H), 6.09 (s, 1H), 2.84 and 2.78 (AB System, *J* = 16.4 Hz, 2H), 2.41 and 2.32 (AB System, *J* = 16.4 Hz, 2H), 1.22 (s, 3H), 1.12 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.1, 164.3, 147.7, 147.2, 142.5, 142.2, 140.7, 140.2, 131.3, 130.2, 129.8, 129.6, 129.2, 128.9, 126.0, 122.2, 120.9, 115.1, 114.2, 51.0, 41.4, 33.7, 32.6, 29.5, 27.8 ppm. HRMS (ESI, m/z): calcd for $C_{33}H_{25}N_3O_2$ ($M+H^+$) 495.195; found: 495.191.

**4.3.13. 3,3-dimethyl-16-(3,4,5-trimethoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4m).** Yield 90%; yellow powder; mp 239-241 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.39 (d, *J* = 7.7 Hz, 1H), 8.39-8.34 (m, 2H), 8.28-8.25 (m, 1H),

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7.86-7.80 (m, 4H), 6.89 (s, 2H), 6.12 (s, 1H), 3.78 (s, 6H), 3.67 (s, 3H), 2.83 (s, 2H), 2.42 and 2.38 (AB System, $J$ = 16.4 Hz, 2H), 1.23 (s, 3H), 1.21 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 164.0, 152.7, 142.3, 141.9, 140.3, 130.3, 130.2, 130.0, 129.2, 129.0, 128.6, 126.6, 125.7, 122.1, 116.2, 114.8, 106.5, 60.7, 56.2, 51.0, 41.3, 33.6, 32.5, 29.9, 27.3 ppm. HRMS (ESI, m/z): calcd for $C_{34}H_{30}N_2O_5$ ($M+H^+$) 546.215; found: 546.208.

**4.3.14. 3,3-dimethyl-16-(thiophen-2-yl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4n).** Yield 90%; yellow powder; mp 259-261 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.31 (dd, $J$ = 2.0, 8.0 Hz, 1H), 8.32-8.29 (m, 2H), 8.26-8.23 (m, 1H), 7.82-7.77 (m, 4H), 7.10 (d, $J$ = 6.9 Hz, 1H), 6.97-6.95 (q, 1H), 6.75-6.73 (m, 1H), 6.50 (s, 1H), 2.83 and 2.72 (AB System, $J$ = 16.4 Hz, 2H), 2.44 (s, 2H), 1.23 (s, 3H), 1.22 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 196.8, 164.4, 148.7, 147.1, 142.6, 141.7, 141.1, 140.9, 130.9, 130.1, 130.0, 129.6, 128.5, 126.5, 125.5, 123.7, 122.1, 115.8, 114.7, 51.1, 41.4, 32.5, 29.7, 28.2, 27.7 ppm. HRMS (ESI, m/z): calcd for $C_{29}H_{22}N_2O_2S$ ($M+H^+$) 462.140; found: 462.130.

**4.3.15. 3,3-dimethyl-16-(3-nitrophenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4o).** Yield 91%; yellow powder; mp 280-282 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.31-9.26 (m, 1H), 8.68 (d, $J$ = 8.6 Hz, 1H), 8.56-8.54 (m, 1H), 8.30-8.25 (m, 2H), 8.21 (d, $J$ = 8.0 Hz, 2H), 8.02-799 (m, 3H), 7.34-729 (m, 2H), 6.15 (s, 1H), 2.87 and 2.80 (AB System, $J$ = 16.2 Hz, 2H), 2.42 and 2.32 (AB System, $J$ = 16.2 Hz, 2H), 1.22 (s, 3H), 1.13 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 196.6, 164.6, 148.3, 147.2, 142.6, 141.5, 140.4, 135.6, 131.3, 129.9, 129.6, 129.3, 128.9, 126.4, 125.6, 124.4, 122.3, 121.6, 114.9, 51.1, 41.5, 34.1, 32.7, 29.5, 27.6 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{23}N_3O_4$ ($M+H^+$) 501.169; found: 501.162

**4.3.16. 16-(3-fluorophenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4p).** Yield 90%; yellow powder; mp 313-315°C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.27 (dd, $J$ = 2.2, 8.9 Hz, 1H), 8.30 (dd, $J$ = 2.2, 9.8 Hz, 1H), 8.19 (dd, $J$ = 2.0, 10.1 Hz, 2H), 7.81-7.72 (m, 4H), 7.36 (d, $J$ = 8.0 Hz, 1H), 7.32-7.28 (m, 1H), 7.12-7.07 (m, 1H),, 6.72-6.67 (m, 1H), 6.08 (s, 1H), 2.80 and 2.74 (AB System, $J$ = 17.6 Hz, 2H), 2.40 and 2.33 (AB System, $J$ = 16.4 Hz, 2H), 1.20 (s, 3H), 1.12 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 196.9, 163.9, 147.1, 142.6, 141.7, 141.0, 140.8, 131.0, 129.7, 129.5, 129.2, 128.6, 126.4, 125.6, 124.8, 122.0, 116.3, 116.1, 115.7, 114.8, 113.4, 51.1, 41.4, 33.6, 32.6, 29.6, 27.5 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{23}FN_2O_2$ ($M+H^+$) 474.174; found: 474.170.

**4.3.17. 3,3-dimethyl-16-(4-nitrophenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4q).** Yield 90%; yellow powder; mp 296-298 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.32-9.30 (m, 1H), 8.38-8.33 (m, 1H), 8.30-8.27 (m, 1H), 8.25-8.20 (m, 1H), 8.17-

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8.13 (m, 1H), 8.06-7.98 (m, 2H), 7.82-7.77 (m, 6H), 6.13 (s, 1H), 2.80 (s, 2H), 2.41 and 2.31 (AB System, $J$ = 16.4 Hz, 2H), 1.22 (s, 3H), 1.10 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 196.8, 164.2, 152.6, 147.2, 146.4, 142.5, 141.5, 141.1, 130.3, 130.1, 129.9, 129.6, 129.2, 128.9, 125.6, 123.3, 122.2, 114.8, 114.1, 50.9, 41.4, 34.3, 32.6, 29.6, 27.4 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{23}N_3O_4$ ($M+H^+$) 501.169; found: 501.162.

**4.3.18. 16-(4-(dimethylamino)phenyl)-3,3-dimethyl-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4r).** Yield 93%; yellow powder; mp 248-250 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.36 (d, $J$ = 5.6 Hz, 1H), 8.39 (d, $J$ = 8.0 Hz, 1H), 8.30-8.27 (m, 1H), 8.20-8.17 (m, 1H), 7.76-7.72 (m, 4H), 7.41 (d, $J$ = 6.9 Hz, 2H), 7.11 (d, $J$ = 6.9 Hz, 2H), 6.12 (s, 1H), 3.08 (s, 6H), 2.85 and 2.78 (AB System, $J$ = 16.4 Hz, 2H), 2.44 and 2.37 (AB System, $J$ = 16.4 Hz, 2H), 1.22 (s, 3H), 1.12 (s, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.2, 164.4, 147.7, 141.9, 140.7, 140.3, 131.3, 130.3, 129.5, 129.3, 128.9, 126.1, 125.7, 122.3, 120.1, 115.1, 114.2, 51.0, 47.1, 41.3, 33.7, 32.6, 29.4, 27.7 ppm. HRMS (ESI, m/z): calcd for $C_{33}H_{29}N_3O_2$ ($M+H^+$) 499.226; found: 499.219.

**4.3.19. 16-(4-ethoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4s).** Yield 90%; yellow powder; mp 224-226 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.35 (d, $J$ = 7.6 Hz, 1H), 8.37 (d, $J$ = 6.9 Hz, 1H), 8.27-8.23 (m, 2H), 7.84-7.76 (m, 4H), 7.48 (d, $J$ = 8.0 Hz, 2H), 6.67 (d, $J$ = 8.0 Hz, 2H), 6.11 (s, 1H), 3.88-3.83 (q, 2H), 3.02-2.95 (m, 1H), 2.89-2.81 (m, 1H), 2.57-2.42 (m, 2H), 2.21-2.12 (m, 2H), 1.27 (t, $J$ = 6.9 Hz, 3H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.2, 165.2, 157.4, 147.0, 142.7, 142.1, 140.7, 137.4, 130.0, 129.9, 129.3, 128.4, 125.6, 122.0, 113.9, 63.2, 37.3, 32.7, 27.7, 20.7, 14.9 ppm. HRMS (ESI, m/z): calcd for $C_{31}H_{24}N_2O_3$ ($M+H^+$) 472.179; found: 472.172.

**4.3.20. 16-(4-isopropylphenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4t).** Yield 95%; yellow powder; mp 250-252 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.31 (dd, $J$ = 1.8, 8.2 Hz, 1H), 8.36-8.33 (m, 1H), 8.26-8.22 (m, 2H), 7.83-7.745 (m, 4H), 7.49 (d, $J$ = 8.0 Hz, 2H), 6.69 (d, $J$ = 8.0 Hz, 2H), 6.15 (s, 1H), 3.01-2.95 (m, 1H), 2.89-2.82 (m, 1H), 2.75-2.68 (m, 1H), 2.55-2.48 (m, 2H), 2.21-2.10 (m, 2H), 1.08 (dd, $J$ = 2.2, 6.6 Hz, 6H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.2, 165.2, 147.0, 146.6, 142.6, 142.4, 142.0, 140.7, 130.7, 130.0, 129.9, 129.6, 129.3, 128.8, 128.3, 126.7, 126.1, 125.6, 122.0, 116.7, 37.3, 33.6, 33.0, 27.7, 24.0, 20.7 ppm. HRMS (ESI, m/z): calcd for $C_{32}H_{26}N_2O_2$ ($M+H^+$) 470.199; found: 470.192.

**4.3.21. 16-(2-chlorophenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4u).** Yield 90%; yellow powder; mp 230-232 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.29-9.26 (m, 1H), 8.32-8.30 (m, 1H), 8.21-8.18 (m, 2H), 7.80-7.75 (m, 4H),7.53-7.47 (m, 2H), 7.10-6.97

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(m, 2H), 6.07 (s, 1H), 3.00-2.96 (m, 1H), 2.87-2.80 (m, 1H), 2.52-2.48 (m, 2H), 2.21-2.14 (m, 2H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 165.6, 147.2, 142.6, 141.9, 141.1, 133.7, 130.1, 129.7, 129.4, 129.2, 129.1, 128.6, 127.7, 126.6, 126.4, 125.6, 122.1, 115.9, 37.2, 33.6, 27.7, 20.6 ppm. HRMS (ESI, m/z): calcd for $C_{29}H_{19}ClN_2O_2$ ($M+H^+$) 462.114; found: 462.110.

**4.3.22. 16-(2-methoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4v).** Yield 91%; yellow powder; mp 302-304 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.28 (dd, *J* = 2.2, 7.9 Hz, 1H), 8.32-8.30 (m, 1H), 8.24-8.20 (m, 2H), 7.80-7.74 (m, 4H), 7.21-7.19 (m, 1H), 7.15 (d, *J* = 6.9 Hz, 1H), 7.06 (t, *J* = 6.9 Hz, 1H), 6.12 (s, 1H), 3.72 (s, 3H), 3.00-2.93 (m, 1H), 2.88-2.80 (m, 1H), 2.57-2.46 (m, 2H), 2.20-2.10 (m, 2H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.1, 165.3, 159.2, 146.9, 146.7, 142.5, 141.9, 140.6, 130.6, 130.0, 129.6, 129.4, 129.3, 128.8, 128.4, 126.5, 125.5, 121.9, 121.5, 116.3, 116.1, 115.4, 111.4, 55.2, 37.2, 33.5, 27.6, 20.6 ppm. HRMS (ESI, m/z): calcd for $C_{30}H_{22}N_2O_3$ ($M+H^+$) 458.163; found: 458.155.

**4.3.23. 16-(3-methoxyphenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4w).** Yield 95%; yellow powder; mp 241-243 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.28 (d, *J* = 7.9 Hz, 1H), 8.36-8.32 (m, 1H), 8.21-8.16 (m, 2H), 7.81-7.73 (m, 4H), 7.08-7.03 (m, 2H), 6.88-6.84 (m, 2H), 6.03 (s, 1H), 3.76 (s, 3H), 3.00-2.94 (m, 1H), 2.87-2.81 (m, 1H), 2.57-2.46 (m, 2H), 2.20-2.11 (m, 2H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.2, 164.8, 147.1, 144.6, 142.7, 142.0, 140.6, 137.6, 130.0, 129.6, 129.5, 128.7, 128.4, 126.2, 125.8, 122.5, 121.5, 116.2, 115.4, 111.4, 56.2, 37.3, 30.2, 27.8, 20.6 ppm. HRMS (ESI, m/z): calcd for $C_{30}H_{22}N_2O_3$ ($M+H^+$) 458.163; found: 458.155.

**4.3.24. 16-(3-chlorophenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4x).** Yield 90%; yellow powder; mp 289-291 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.29-9.27 (m, 1H), 8.33-8.31 (m, 1H), 8.21-8.17 (m, 2H), 7.82-7.75 (m, 5H),7.37 (d, *J* = 8.0 Hz, 1H), 67.29-7.24 (m, 1H), 7.12-7.01 (m, 1H), 6.73-6.68 (m, 1H), 6.11 (s, 1H), 3.00-2.94 (m, 1H), 2.89-2.82 (m, 1H), 2.58-2.44 (m, 2H), 2.22-2.10 (m, 2H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 165.5, 147.2, 147.0, 142.5, 141.7, 140.8, 140.6, 133.8, 131.1, 130.8, 130.5, 130.1, 129.7, 129.4, 129.2, 129.1, 128.6, 127.8, 127.7, 126.6, 126.4, 125.5, 125.1, 122.0, 115.9, 115.5, 37.2, 33.6, 27.7, 20.6 ppm. HRMS (ESI, m/z): calcd for $C_{29}H_{19}ClN_2O_2$ ($M+H^+$) 462.114; found: 462.118.

**4.3.25. 16-(3-fluorophenyl)-2,3,4,16-tetrahydro-1H-benzo[a]chromeno[2,3-c]phenazin-1-one (4y).** Yield 92%; yellow powder; mp 285-287 °C. $^1$H-NMR (400 MHz, $CDCl_3$): δ 9.33 (dd, *J* = 1.9, 7.6 Hz, 1H), 8.38-8.25 (m, 3H), 8.16-8.14 (m, 1H), 7.86-7.67 (m, 3H),7.44 (d, *J* = 8.0 Hz, 2H), 6.68 (d, *J* = 8.0 Hz, 2H), 6.11 (s, 1H), 3.1-2.95 (m, 1H), 2.91-2.85 (m, 1H), 2.52-2.48 (m, 2H), 2.22-2.10 (m, 2H) ppm. $^{13}$C-NMR (100 MHz, $CDCl_3$): 197.0, 165.5, 162.5 (d, *J* = 240.2

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Hz), 147.7, 147.0, 142.5, 141.7, 141.0, 140.8, 130.9, 130.0, 129.6, 129.5, 129.2, 128.5, 126.4, 125.5, 124.8, 122.0, 116.2, 116.0, 115.7, 113.3, 37.2, 33.5, 27.7, 20.6 ppm. HRMS (ESI, m/z): calcd for $C_{29}H_{19}FN_2O_2$ ($M+H^+$) 446.143; found: 446.135.

### 4.4. Biological assays

#### 4.4.1. DPPH radical scavenging activity

The DPPH gives a strong absorption maximum at $\lambda$= 517 nm (purple colour) with nitrogen centered stable free radicalthat is suitable for spectrophotometric studies. For the dioxane/ethanol solution (100 μM) of DPPH the test compounds in solutions (100 μM) were added. The absorbance was measured at $\lambda$= 517 nm after 20 min kept the tubes at ambient temperature. To express the % scavenging of DPPH radical using **Eq.-1.**

#### 4.4.2. Hydrogen Peroxide ($H_2O_2$) Scavenging Activity

The compound's $H_2O_2$ scavenging activity was determined using a 40 mM solution of peroxidein phosphate buffered saline (50 mM, PBS, pH 7.4). To the solution of $H_2O_2$-PBS (0.6 mL) added 100 μM compound solution (in 4 mL distilled water). Absorbance of analyte mixturewas determined at $\lambda$= 230 nmon spectrophotometer after 10 min against a blank solution, which isparent compound with PBS and without $H_2O_2$. Onthe same way standard compound, Vitamin-C, took in place of test compound and observed the absorbance after 10 min against a blank solution.

#### 4.4.3. Reducing Power

In reducing power assay method the test compounds (at 25, 50, 75, and 100 mg/mL concentrations in methanol) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassiumferricyanide [$(K_3Fe(CN)_6$] (1% w/v)to forms coloured complexes.2.5 mL of trichloro acetic acid (TCA, 10% w/v) wasadded after incubating the resulting mixture for 20 min at 50 °C. After 10 min centrifugation of the mixture at 3000 rpmwas added 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%, w/v) to the upper layer of the solution (2.5 mL) and determined the UV absorbance on spectrophotometerat 700 nm against blank sample. For each compound used three independent samples to measure the mean values and was found less than 2% standard deviations (SD).

#### 4.4.4. Anticancer activity

**Cell culture**

Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide (MTT) assay. T-75 tissue culture flasks (Nunc, Denmark) was used to culture theHeLa

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cells at 37 °C in a 5% $CO_2$ humidified incubator using appropriate media supplemented with DMEM containing 10% heat-inactivated FBS. Similarly, RPMI media was used to culture the SK-BR-3 cells. Under identical conditions each wellwith 100 µL medium in 96 well microtiter plate were seeded the cells at a final density of $2 \times 10^4$ cells/well. In different concentrations (0.1–100 µg/mL) or DMSO (carrier solvent) of the test compounds were used to treat the cells after overnight incubation with 3 replicates each in a final volume of 200 µL. 10 µL of MTT (5 mg/ mL) was added after 24 h to each well and the plate was incubatedat 37 °C for 4 h in the dark. The MTT and mediawere removedand the formazan crystals solubilized in DMSO (100 µL/well). Finally, MTT reduction was measured by measure the absorbance at $\lambda$= 570 nm using GENios® microplate reader (Tecan Austria GmbH, Austria). By addition of untreated cells with DMSO as controlmeasured the effects of the tilted compounds on cell viability. The regression lines were plotted for the best straight-line fit of the data to linear regression analysis were subjected.The concentrations at which 50% of the cells are dead ($IC_{50}$) were calculated using the respective regression equation.

**Supporting Information**

Electronic supplementary information (ESI) available: Spectral data and copies of $^1$H-NMR and $^{13}$C-NMR spectra for all the compounds synthesized

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# Tetramethylguanidiniumchlorosulfonate ionic liquid (TMG IL): An efficient reusable catalyst for the synthesis of tetrahydro-1*H*-benzo[*a*]chromeno[2,3-*c*]phenazin-1-ones under solvent-free conditions and evaluation for their *in vitro* bioassay

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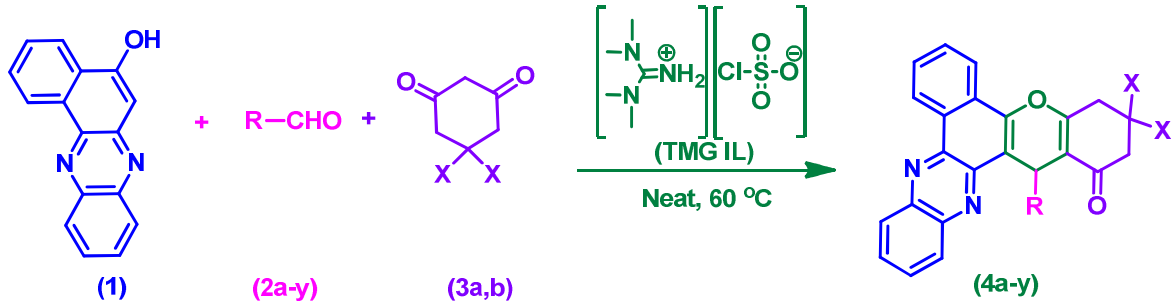


The scope and versatility of the green catalytic multi-component reaction has been demonstrated in this methodology are highly reactive, potent activity, ecologically cleaner route and reusability.