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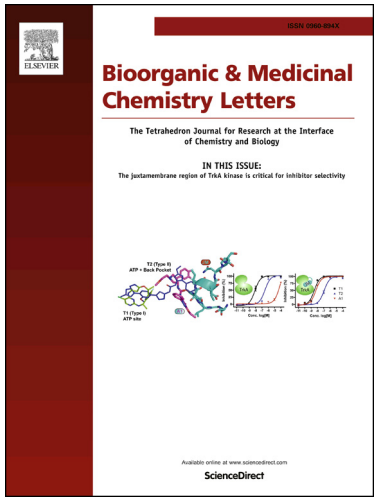

Expanding the Tetrahydroquinoline Pharmacophore

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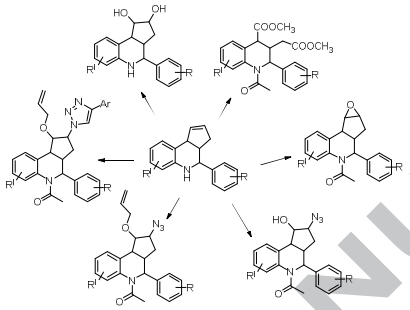

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Expanding the Tetrahydroquinoline Pharmacophore

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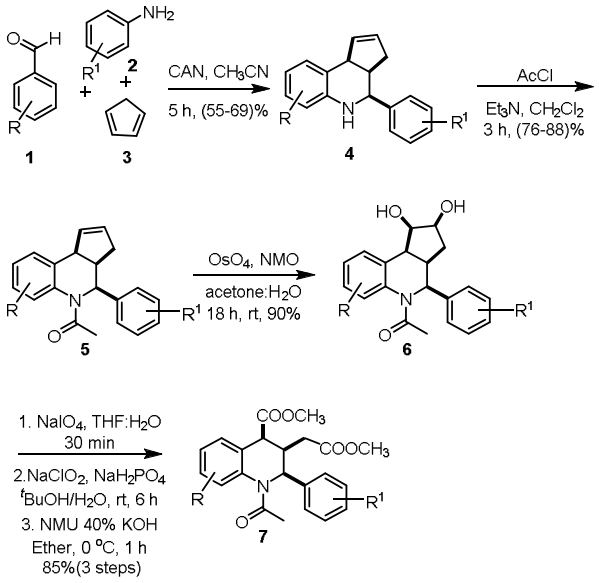
ABSTRACT

Tetrahydroquinoline is a privileged scaffold with a large number of biological applications. The tetrahydroquinoline pharmacophore has been expanded to yield 34 compounds. Biological screening of these compounds led to the identification of tetrahydroquinoline as neurotropic agents not reported earlier.

Tetrahydroquinoline is a privileged scaffold in HIT identification and has shown activity in a variety of assays. Compounds having tetrahydroquinoline skeleton are shown to be antibacterials,[1a] antitumor agents,[1b] HIV protease inhibitors,[1c] CRTH2 inhibitors for treatment of allergic diseases triggered by prostaglandins,[1d] activators of cannabinoid receptors acting as antiemetics and analgesics,[1e] compounds altering lifespan of eukaryotes[1f] or imaging G-protein coupled estrogen receptors.[1g] In addition, they find applications as ligands for Rh-catalyzed asymmetric hydrogenations,[2a] asymmetric Friedel-Craft's reactions,[2b] as dyes,[2c-f] antioxidants[2g] and corrosion inhibitors.[2h] These varied activities have prompted researchers to explore different synthetic methods to obtain tetrahydroquininline derivatives in optimal yields and purity. One such reaction is Povarov reaction or *aza* Diels-Alder reaction which is catalyzed using a wide range of acids, both Lewis and Bronsted, and include bismuth (III) bromide,[3a,b] niobium (V) chloride,[3c] samarium iodide,[3d] cerium (IV) ammonium nitrate,[3e] magnesium perchlorate,[3f] copper (II) bromide,[3g] indium trichloride,[3h-k] lanthanum reagents,[3l-n] $BF_3.Et_2O$,[3o] $FeCl_3$[3p] *etc*. The reaction has also been carried out using clay/water mixtures[4a].

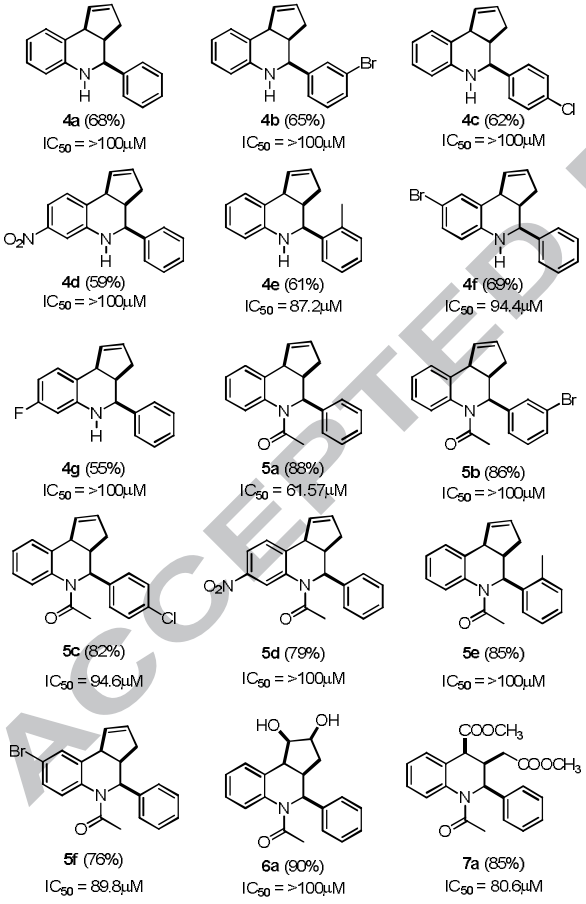
The synthesis of tetrahydroquinolines and identification of hits such as WAY-163909 and vebicaserin as $5HT_{2c}$ antagonists[5] and screening of diversity oriented synthesis of these scaffolds as antitubercular agents[6] are reported recently. Most of the publications in this area report the synthesis of the tetrahydroquinoline scaffold and explore its biological activity. There are no reported attempts to further explore the scaffold by modifying the rings. In our endeavor to identify new scaffolds for drug discovery, we synthesized a library of **34** tetrahydroquinoline compounds based on the *aza* Diels-Alder reaction and these were screened for neurotropic activity. A detailed account of this work is presented here.

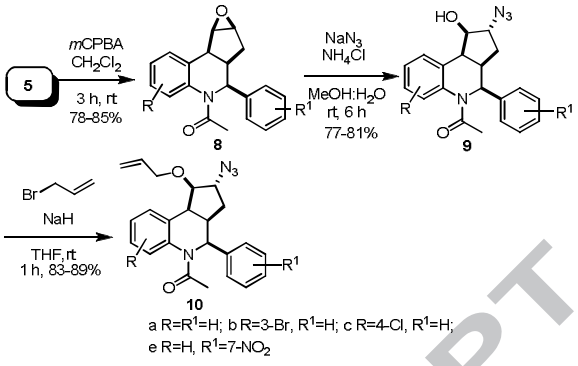
The first step in the library synthesis started with the *aza* Diels-Alder reaction. The reaction was carried out using aniline, benzaldehyde and cyclopentadiene catalyzed by cericammonium nitrate (CAN). The substituted tetrahydroquinoline **4a** was obtained in 68% yield. Acetylation of **4a** gave **5a** (88% yield) which on reaction with osmium tetroxide yielded diol **6a** in 90% yield. Treatment of diol with sodium periodate opened the cyclopentane ring to give dialdehyde which was immediately converted to diacid and diester **7a** in overall yield of 85% over 3 steps. Thus a series of six simple chemical conversions resulted in four new compounds with tetrahydroquinoline skeleton (Scheme 1). The sequence of reactions was repeated for substituted tetrahydroquinolines resulting in a library of 15 new compounds. (**4a-4g, 5a-5f, 6a, 7a**).



a R=R^1=H; b R=3-Br, R^1=H; c R=4-Cl, R^1=H; d R=H, R^1=7-NO$_2$
e R=2-Me, R^1=H f R=H, R^1=8-Br; g R=H, R^1=F

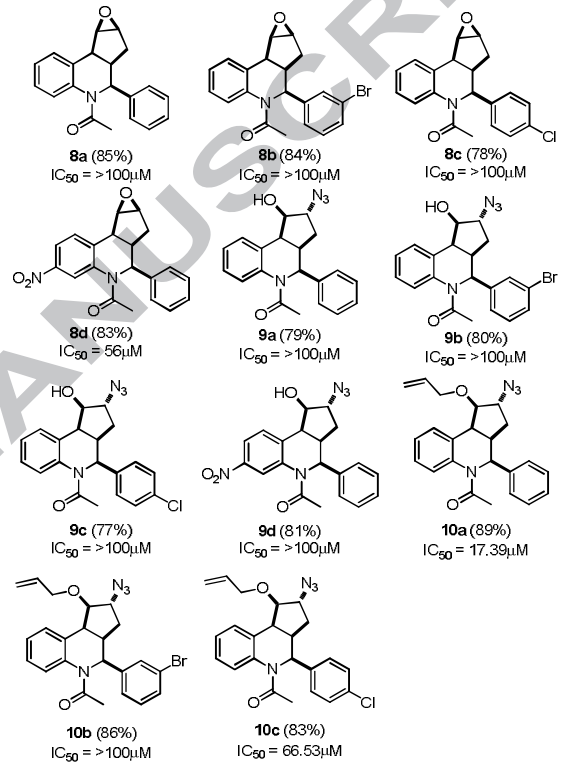
Scheme-1





a R=R^1=H; b R=3-Br, R^1=H; c R=4-Cl, R^1=H;
e R=H, R^1=7-NO$_2$

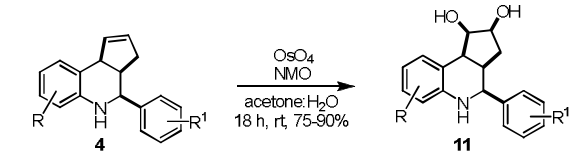
Scheme-2



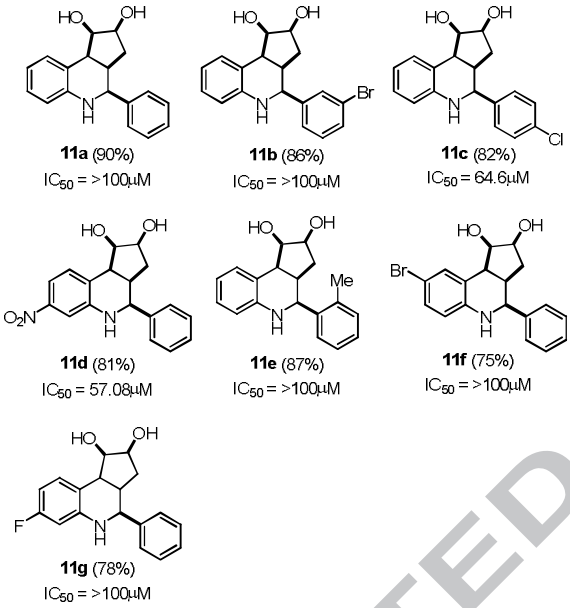
The acetylated compound **5a** was converted, in another sequence, to epoxy derivative **8a** in 85% yield which on reaction with sodium azide and ammonium chloride yielded the azido derivative **9a** in 79% yield. Synthesis of *O*-allyl ether **10a**, was carried out with allyl bromide and sodium hydride in 89% yield (Scheme 2). Substituted tetrahydroquinoline derivatives were also synthesized following the same sequence. (**8a-8e, 9a-9e, 10a-10c**)

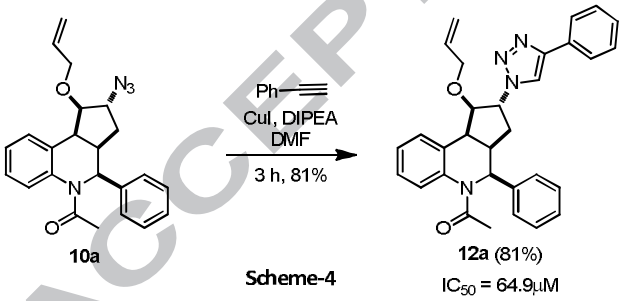
The tetrahydroquinoline **4a** was reacted with osmium tetroxide in presence of NMO to get diol **11a** in 90% yield (Scheme 3). In addition, other diols **11b-e** were synthesized following same sequence. The *O*-allyl ether, **10a**, was treated with phenylacetylene in presence of copper (I) iodide to get 1,2,3- triazole **12a** in 81% yield (scheme 4).

All these compounds had the tetrahydroquinoline skeleton undisturbed with functional groups that can help extend the library further. To study the properties of these compounds, they were subjected to biological screening. As reported[6] earlier, these scaffolds were expected to be anti-tubercular compounds. Screening of these compounds against *Mycobacterium bovis* (scheme **1-4**) did not show any significant activity.



Scheme-3



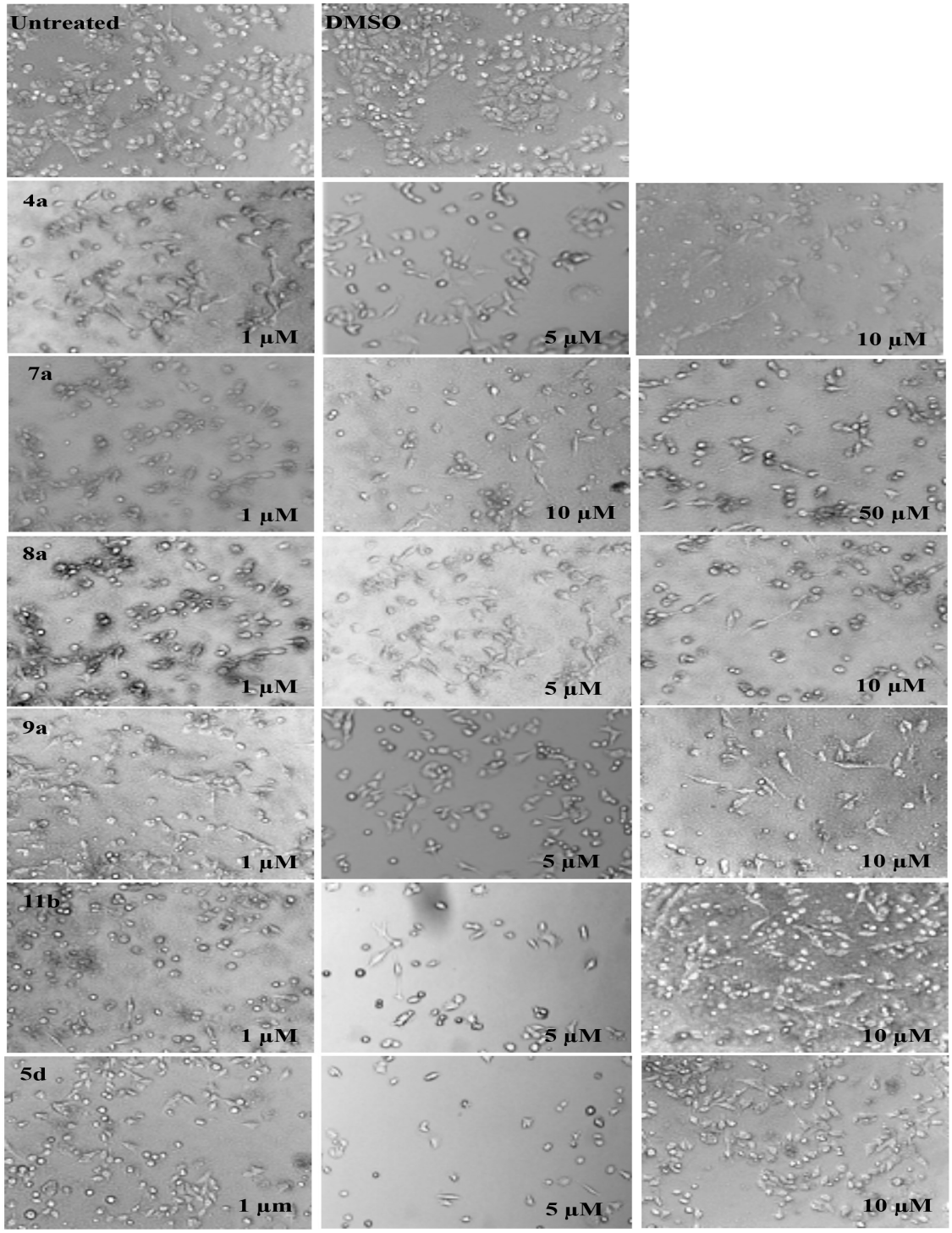


Scheme-4

The whole library of compounds was then screened for other biological activity. Primary screening of the compounds was carried out against proliferation of a variety of cancer cell lines such as A 549, DU 145, MCF 7, MDM2 and Hep G2. The present series of compounds did not exhibit any cytotoxic effect against the cell lines tested. However, some compounds showed neurotropic activity with no cytotoxicity when added to IMR-32 neuroblastoma cells. From the results obtained it was found that all the compounds showed IC_{50} values higher than 50 μM. The compounds with neurotrophic activity displayed IC_{50} values above 100 μM, highlighting no cytotoxicity towards IMR-32 cells. Of all the compounds, **8a** showed an IC_{50} value of 17.39 μM ± 1.84 (Table 1) against IMR-32 cells. Thus, the non-cytotoxic compounds against IMR-32 cells were analyzed for their neurotrophic potential *in vitro*. In order to do so, the cells were treated with compounds at increasing concentrations and allowed to undergo differentiation. Following 96 h of incubation in a 5% CO_2 incubator, the change in morphology was observed at different concentrations. It is known[7] that differentiation of neuroblastoma cells is accompanied with a change in morphology in a time dependent manner. Following differentiation, the development of neuronal morphology is manifested by appearance of elongated neurites in IMR-32 cells. Thus, to ascertain whether the compounds can cause neuronal differentiation, characteristic features of neuronal morphology including the changes in cell morphology and appearance of neurites were observed in IMR-32 cells treated with test compounds. From the results obtained, it was found that compounds **4a, 5d, 7a, 8a, 9a** and **11b** caused a change in morphology of cells after 96 h of treatment. The resultant morphology was similar to neuronal morphology wherein the appearance of neurites was clearly visible (Figure 1a). The average neurite length shows the concentration dependent activity of the test compounds (Figure 1b). This observation further led us to assess the expression of neuronal markers like Brain Derived Neurotrophic Factor (BDNF) and Nerve Growth Factor (NGF). BDNF is a protein whose expression warrants the differentiation of neuroblastoma cells. It is known[8] that BDNF increases survival of neurons and plays a role in promotion of differentiation. NGF is another known neurotrophic factor[9] and its expression is an indicator of neuronal differentiation of neuroblastoma cells. Thus, in order to confirm whether the compounds are inducing differentiation in IMR32 cells, transcript and protein level expression of BDNF and NGF was analyzed. The expression of both the markers is significantly increased at both mRNA and protein level in differentiated IMR-32 cells by test compounds compared to mock treated cells. The fold change in expression at different time points is very well correlating with change in morphology of IMR-32 cells and also length of the neuritis outgrown. It was observed that when cells were treated with compound **4a** at increasing concentrations, differentiation was initiated at 1 μM and the neuronal morphology was evident at 10 μM (Figure 1a & b). The expression of both BDNF and NGF was found to be increased in cells treated with **4a** at 10 μM when compared to DMSO treated cells (Figure 2, 3a & b). Similarly, compounds **5d, 7a, 8a, 9a** and **11b** also showed clear differentiation at 10 μM concentration which was confirmed by increase in expression of BDNF and NGF at both gene and protein levels. Although, compound **7a** induced differentiation at 10 μM concentrations, the neuronal morphology was more pronounced at 50 μM concentration of compound (Figure 2, 3a & b). Details of the biological screening are compiled in Table-1. Below mentioned are the primer sequences used to measure expression of NGF and BDNF in differentiated IMR-32 cells.

S. No	Gene Name	Gene ID	Product size(bp)	Forward& Reverse Primers	Binding site on template
1	GAPDH	NM_001289746.1	496	CAAGGTCATCCATGACAACTTTG	657
				GTCCACCACCCTGTTGCTGTAG	1152
2	BDNF	NM_170735.5	147	CTACGAGACCAAGTGCAATCC	1684
				AATCGCCAGCCAATTCTCTTT	1774
3	Nerve growth factor (NGF)	NM_002506.2	158	GTCATCATCCCATCCCATCT	532
				AGTACTGTTTGAATACACTGTTGTTAAT	689

(a)



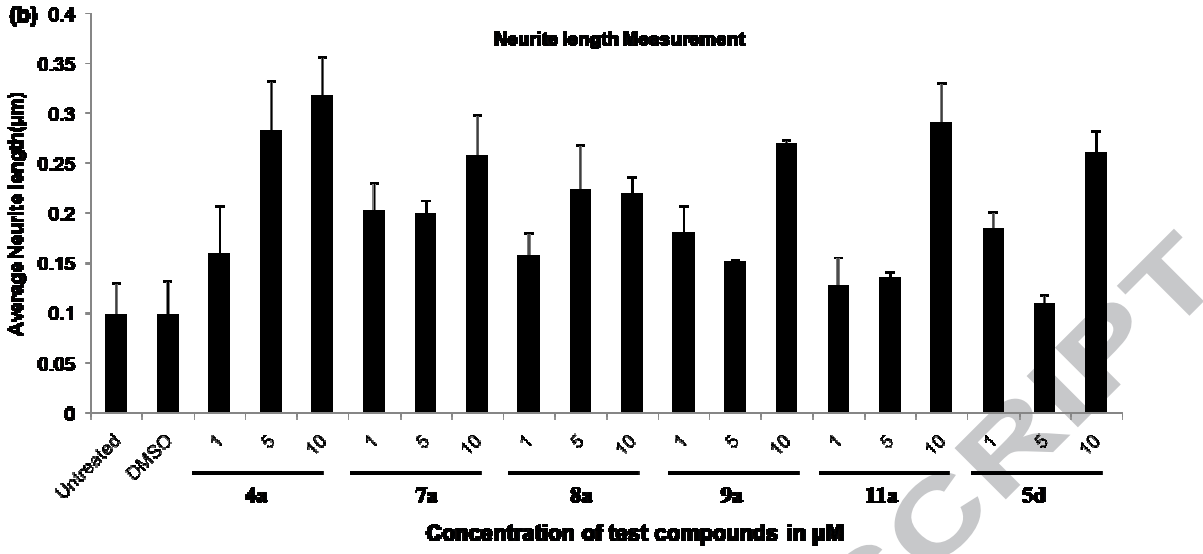


Figure 1: (a) Differentiation and appearance of neuronal morphology of IMR-32 cells treated with respective compounds at increasing concentrations. Untreated cells and DMSO mock treated cells were taken as controls of undifferentiated cells. Only representative images are shown here. (b). Length of neurites outgrown from IMR-32 cells treated with test compounds was measured using Image J program and the length of the neuritis was expressed in micro meters.

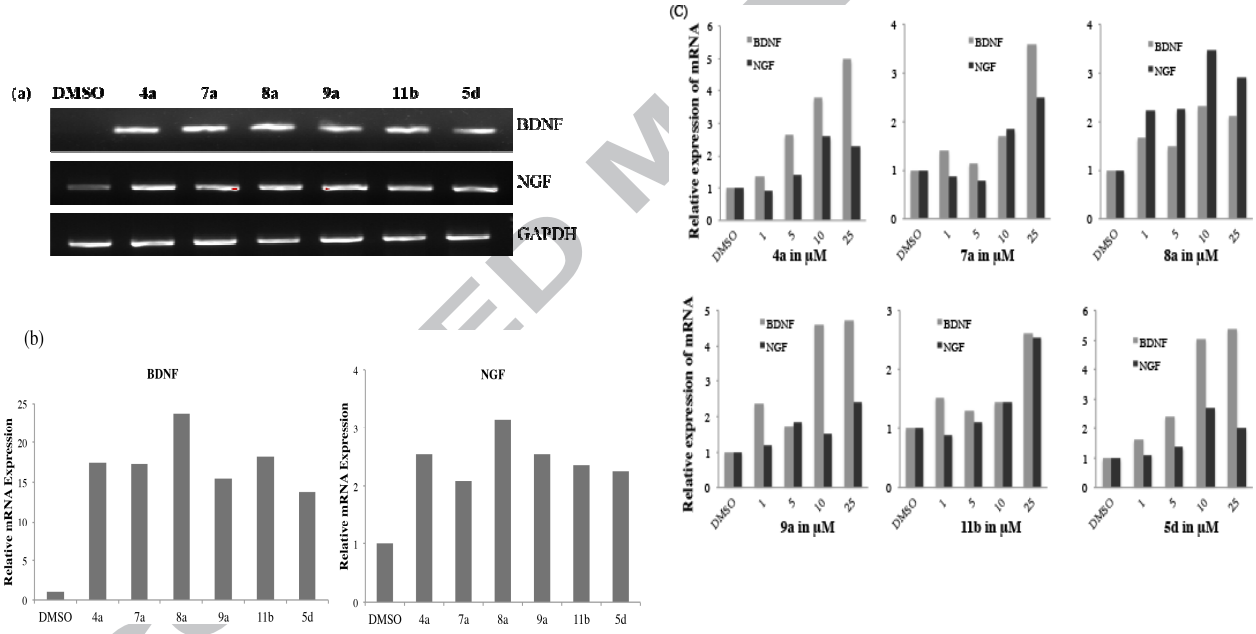


Figure 2: (a) Gene expression of neurotrophic factors BDNF and NGF in cells treated with respective compounds at a concentration of 10µM. (b) Densitometry of the RT PCR results to determine fold change in the expression of transcripts in IMR-32 cells. (c) Measuring gene expression by quantitative RT-PCR to determine concentration dependent (1 to 25µ**M**) induction of NGF and BDNF gene expression. Depending on the concentration of test compound the relative fold increase in expression of both the genes was correlated. GAPDH expression was measured as housekeeping gene to calculate relative fold changes in gene expression.

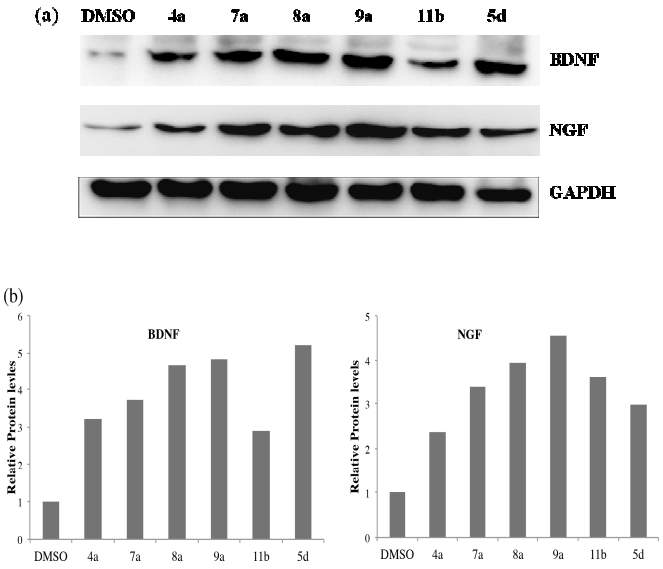


Figure 3: (a) Protein expression of neurotrophic factors BDNF and NGF in cells treated with respective compounds at a concentration of 10μM. GAPDH expression was measured to ensure equal loading protein from different samples.is considered as a constitutive control. (b) Densitometry of the blots showing fold change in relative expression of proteins upon differentiation by test compounds.

		Cytotoxicity against IMR32			Neurotrophic activity in IMR32
S.No	MTP No.	IC_{50} (μM)	STD DEV	Remarks	Concentration (μM)
1	4a	>100		NC	Neurotrophic at10 μM
2	5a	61.57	3.65	CT	NN
3	7a	80.60	1.24	CT	Neurotrophic at50 μM
4	8a	>100		NC	Neurotrophic at10 μM
5	9a	>100		NC	Neurotrophic at10 μM
6	10a	17.39	1.84	CT	NN
7	11a	>100		NC	NN
8	4d	>100		NC	NN
9	4c	>100		NC	NN
10	5c	94.67	2.56	CT	NN
11	11c	64.59	2.60	CT	NN
12	8c	>100		NC	NN
13	9c	>100		NC	NN
14	10c	66.54	1.26	CT	NN
15	4b	>100		NC	NN
16	11b	>100		NC	Neurotrophic at10 μM
17	8b	>100		NC	NN
18	9b	>100		NC	NN
19	10b	>100		NC	NN
20	5d	>100		NC	Neurotrophic at10 μM
21	8d	55.99	2.35	CT	NN
22	9d	>100		NC	NN
23	11d	57.08	0.29	CT	NN
24	4e	87.21	2.37	CT	NN
25	11e	>100		NC	NN
26	4f	94.37	0.14	CT	NN
27	11f	>100		NC	NN
28	5f	89.86	0.96	CT	NN
29	4g	>100		NC	NN
30	11g	>100		NC	NN
31	12a	64.96	3.42	CT	NN
32	5b	>100		NC	NN
33	5e	>100		NC	NN
34	6a	>100		NC	NN

Table 1- Complete analysis of compounds in the series to determine the cytotoxic activity (IC_{50} values±STD DEV) and the neurotrophic activity of compounds. Doxorubicin was taken as positive control for cytotoxicity against IMR32 cell line and showed an IC_{50} value of 8.49±0.04. **NOTE**: **NC**-Non Cytotoxic, **NN**-Non Neurotrophic, **CT**-Cytotoxic.

Conclusions

Thus, a new application has been identified for the tetrahydroquinoline skeleton which is known to have antitubercular activity. An extension of the tetrahydroquinoline scaffold has generated 34 new compounds which have handles for further derivatisation. These compounds, when screened for neurotrophic activity, showed considerable promise. This opens up a new application area for these compounds.

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