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Identification of new hit to lead magmas inhibitors as potential therapeutics for glioblastoma

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Abstract

In continuation of our previous efforts for the development of potent small molecules against brain cancer, herein we synthesized seventeen new compounds and tested their anti-gliomapotential against established glioblastoma cell lines, namely, D54MG, U251, and LN-229 as well as patient derived cell lines (DB70 and DB93). Among them, the carboxamide derivatives, **BT-851** and **BT-892** were found to be the most active leads in comparison to our established hit compound BT#9.The SAR studies of our hit **BT#9** compound resulted in the development of two new lead compounds by hit to lead strategy. The detailed biological studies are currently underway. The active compounds could possibly act as template for the future development of newer anti-glioma agents.

Keywords

Anticancer; Anti-glioma agents; Hit to lead; Carboxamides; Glioblastoma; Magmas protein; Oxadiazoles; Oxadiazborole

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2023.129330.

Introduction

Glioblastoma (GBM) is a fatal aggressive brain tumor, which has a severe prognosis in spite of the first line chemotherapeutic drugs, surgery and radiation therapies.[1,2] There is no effective second-line treatment at the time of recurrence. The patients' survival is one to two years, with less than 5–10% of people living longer than five years.[3,4] If the cancer is left untreated, survival is classically about three months. The failure of current therapies has multiple causes, including dysfunctional blood brain barrier, heterogeneous cell populations (including glioblastoma stem cells) which leads to treatment resistance and the risk of toxicity to the normal brain3. Consequently, there is an urgent need for the development of newer anti-glioblastoma agents because of dysfunctional blood brain barrier, development of treatment resistance and lack of effective treatment at the time of recurrence.[5–7]

Magmas is a J-like protein found in the mitochondrial matrix. It has been shown to be upregulated in several cancers including glioblastoma (GBM) and increasedexpressionhas been observed to have a protective effect against reactive oxygen species (ROS) mediated apoptosis in different cancers.[8,9] In mammals, Magmas can form complexes with DnaJC19 and DnaJC15, J-protein subunits of the TIM23 (translocase of the inner membrane) trafficking complex.[10] Magmas plays a crucial role in the survival of glioma and consequently Magmas inhibition may possibly be a prominent tool in the treatment of GBM patients.

Previously, we have synthesized a series of small molecule inhibitors and tested their anti-glioma potential.[8,11] Delightfully, one of our hit compound BT#9, significantly exerted anti-cancer effect in glioma *in vitro by* inhibiting cell division, stimulating apoptosis, obstructing cell migration and invasion. Our outcomes also revealed that our hit compound BT#9, could probably cross the blood brain barrier and has a remarkable potential to be developed as a therapeutic lead.[5,12]

Inspired by having a promising hit (BT#9) in hand(Fig. 1), and in continuance of our efforts towards the development of small molecule inhibitors,[13–15] we were prompted to synthesize new lead compounds by hit to lead strategy using Lipinski rule of five and other preclinical criteria.[16–18] Consequently, the authors of this manuscript report the hit-to-lead optimization of BT#9 by synthesizing new BT#9 analogs and exploring the structure activity relationship (SAR) studies (Fig. 1). The guanidine part of BT#9 was interchanged by carboxamides, oxadiazoles and/or oxadiazaboroles. Herein, we have synthesized seventeen compounds and evaluated their anticancer effect on different GBM cell lines.

The synthesis of acid (**5**) is represented in Scheme 1. It was prepared according to our previous reported procedures.[19,20] Initially, β-cyclocitral (**1**) was subjected to Grignard reaction by treating with methyl magnesium bromide with dry THF to yield alcohol (**2**) as yellow oil (Scheme 1). The alcohol gave satisfactory spectral data and was directly converted to wittig salt (**3**) by treatment with triphenylphosphinehy-drobromide in acetonitrile at 90 °C for 12 h. Recrystallization of (**3)** from hexane gave a white crystalline solid. Next, the witting salt was treated with methyl 4-formylbenzoate in DMF in the presence of Sodium tertbutoxide at room temperature for 24 h to furnish the precursor acid

(**5**). Finally, the desired carboxamides were prepared in good yields by treating the acid (**5**) with corresponding amines in the presence of CDI, DMAP in Dimethylformamide at 90 °C for 16 h.

Next, our goal was to synthesize oxadiazole derivatives from the acid (**5**) The synthesis of oxadiazole derivatives was accomplished by a known protocol[21,10] is illustrated in Scheme 2. These compounds were synthesized by an amide coupling strategy by heating substituted commercially available amidoximes **8**(**a**-**e**) and acid (**5**) in the presence of CDI in DMF for 20 h. After purification by silica-gel chromatography (Hexanes/EtOAc: 3:1) oxadiazole derivatives were obtained as solids in good yields.

Consequently, we were prompted to replace the acid functionality with cyano, amidoxime and oxadiazaborole to study the SAR. The synthetic Scheme is illustrated in Scheme 3. The witting salt (**3**) was treated with 4-cyanobenzaldehyde (**10**) in DMF in the presence of Sodium tertiarybutoxide at room temperature for 24 h to furnish **BT-852.**[14] Next, the cyano compound, **BT-852** was straightforwardly converted to amidoxime derivative, **BT-853**, by heating it with Hydroxylamine Hydrochloride in the presence of a base (DIPEA) in ethanol for 18 h.[13] The next step, boron insertion was accomplished by heating **BT-853** with phenyl boronic acid (**13**) in dry toluene in the presence of molecular sieves for 20 h at 110 °C to obtain the desired oxadiazoborole derivative, **BT-854**.[22] All the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS data.

The synthesized compounds were screened against established glioblastoma cell lines, namely, D54MG, U251, and LN-229 as well as patient derived cell lines (DB70 and DB93) to assess cell viability using MTT assay and XTT assays.[23] IC_{50} value of the synthesized compounds are represented in Table 1. In addition partition coefficient values (log P and C logP) have been calculated theoretically and are represented in Table 1.

Among all the tested compounds, amide derivatives, **BT-851** containing benzothiazole motif and **BT-892** containing trifluoromethyl (-CF_3) substitution exhibited significant cytotoxicity withIC_{50} values of 6.6 μM and 3.8 μM, respectively, against D54MG cellline (Table 1). In addition, **BT-892** showed significant cytotoxicity with IC_{50}value of 6.0 μM against U251 cell line in comparison **BT#9** (IC_{50}value of 5.0 μm). Moreover, **BT-892** demonstrated significant cytotoxicity displaying IC_{50}value of 5.9 μM against LN-229 cells, superior to our previous hit compound **BT#9** with IC_{50}value of 6.5 μm (Table 1; Fig. 2).Fascinatingly, one of the active compounds (**BT-851**) exhibited potent activity with IC_{50}value of 6.6 μM and 3.5 μM against patient-derived cell lines DB70 and DB93, respectively (Table 1; Fig. 2).

The biological results revealed that **BT-851** containing benzothiazole motif and **BT-892** containing (-CF_3) substitution emerged as the most active lead compounds against glioma cell lines. Motivating results in patient-derived cell lines provided useful insight about their clinical implications for therapeutic use. The detailed biological studies are under progress.

In conclusion, we have synthesized seventeen-17 new compounds and tested their anticancer effect in comparison to our previously identified hit compound BT#9 against established glioblastomacell lines, namely, D54MG, U251, and LN-229 as well as patient-derived cell

lines (DB70 and DB93). Among them, **BT-851** and **BT-892** emerged as potential lead compounds in D54MG and U251 cell lines. Gratifyingly, **BT-851** demonstrated potent activity against patient derived cell lines (DB70 and DB93) supporting their clinical applications in the future. The detailed biological studies are currently underway. The SAR studies of our hit BT#9 compound resulted in the development of two new lead compounds by hit to lead strategy. The active compounds could possibly act as template for the future development of newer anti-glioma agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Data availability

Data will be made available on request.

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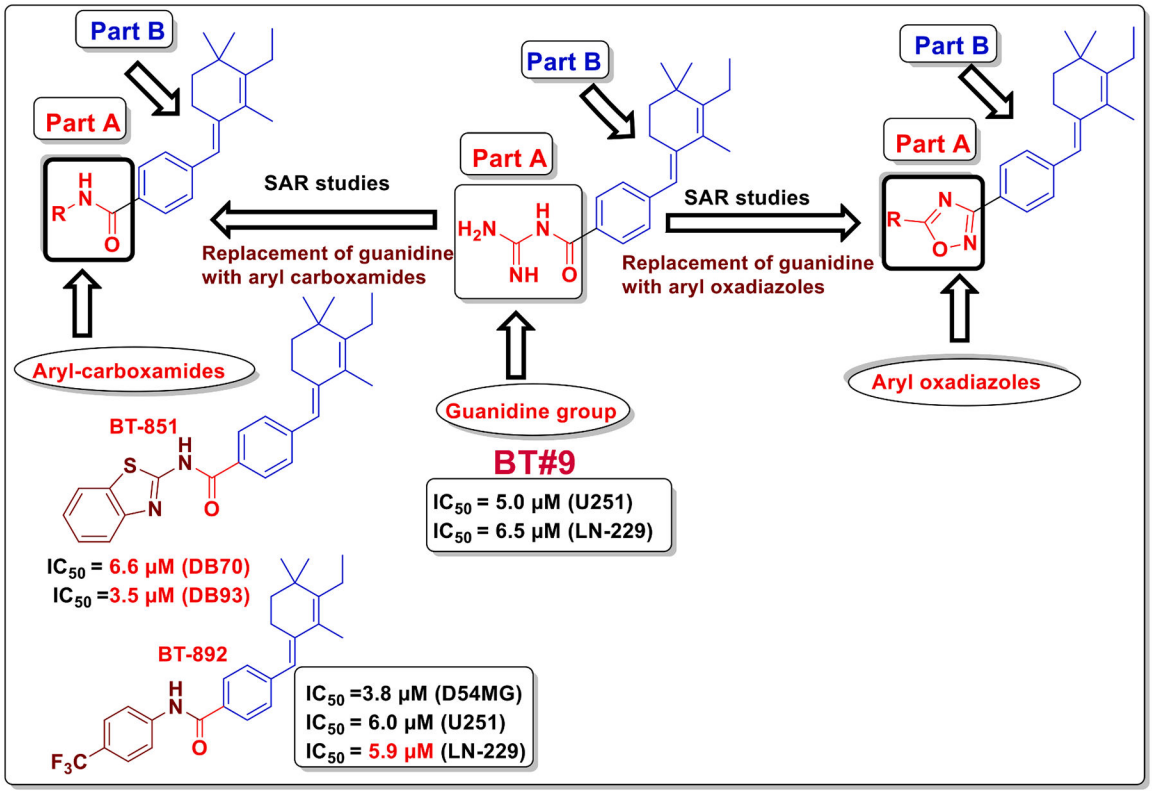


Fig. 1.
SAR studies of BT#9 and identification of two lead compounds BT-851 and BT892.

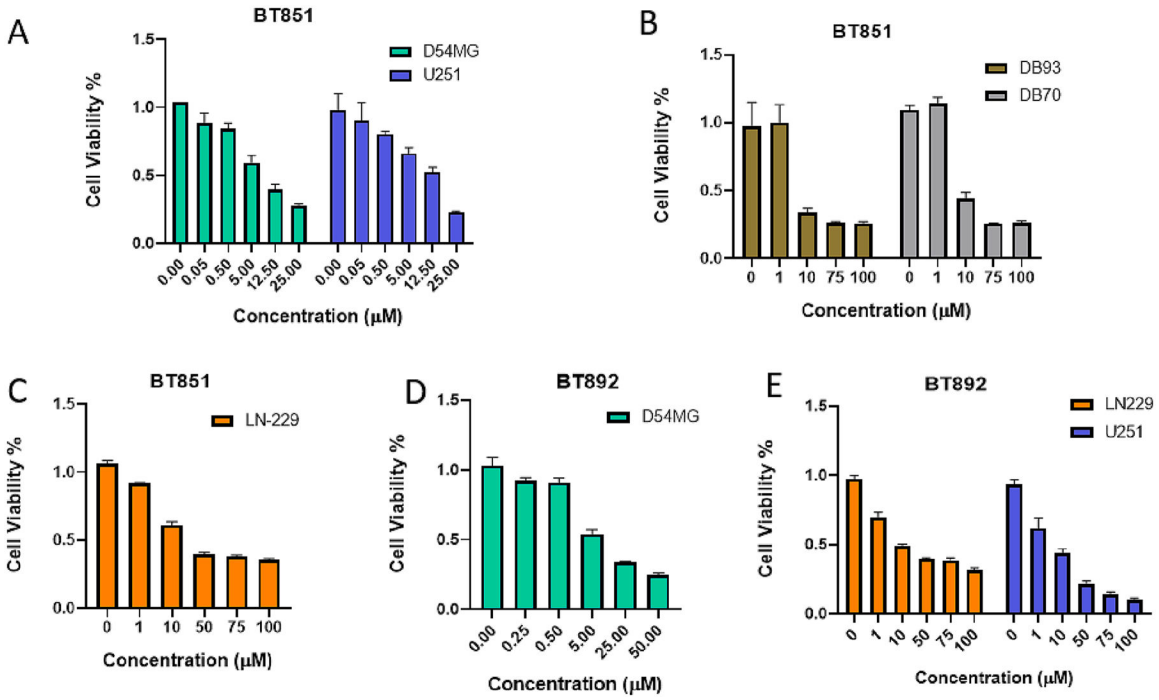
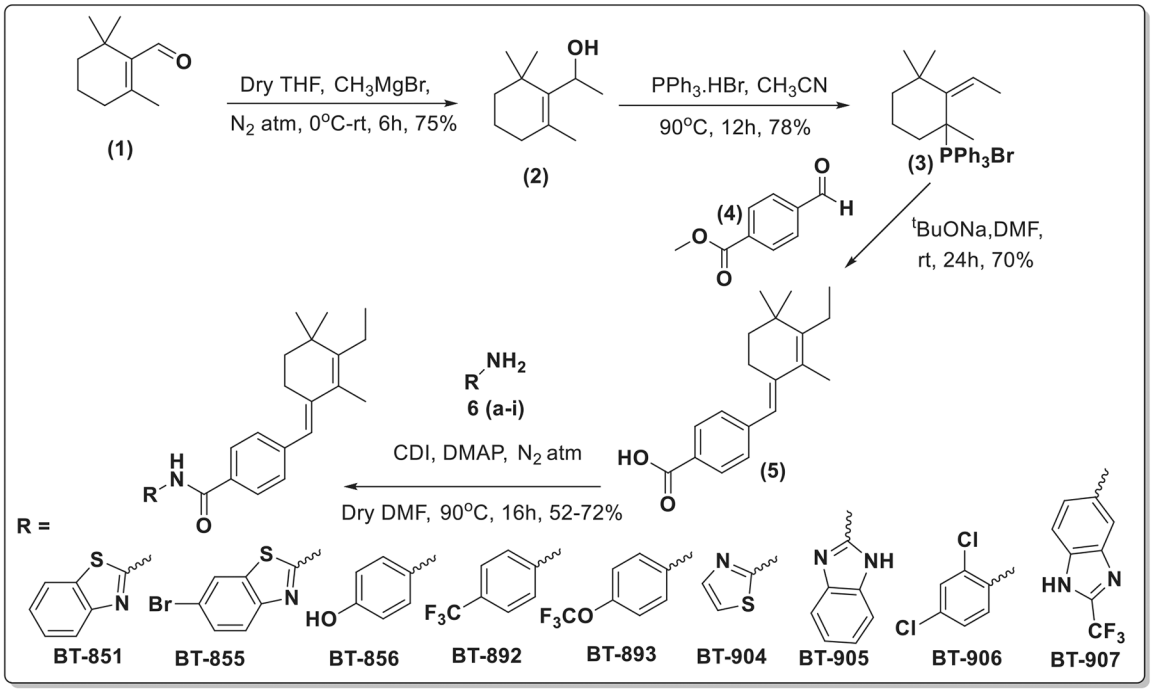
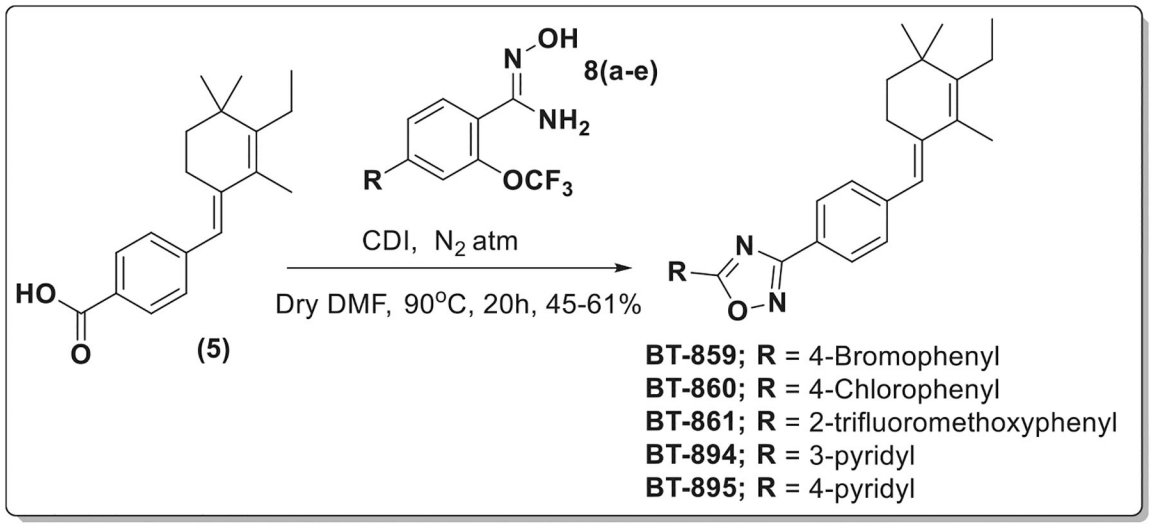


Fig. 2.

Cell viability of established and primary patient GBM cells after inhibitor treatment. Cells were seeded overnight and treated the following day with indicated inhibitors for 3 days.

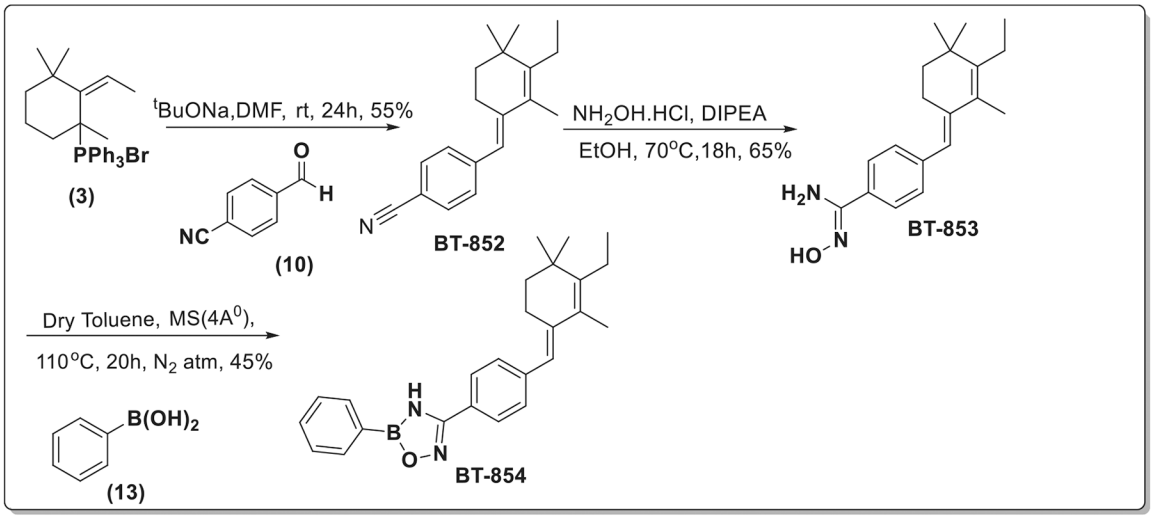


Scheme 1.
Synthesis of carboxamide derivatives.



Scheme 2.

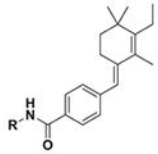
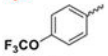
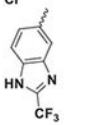
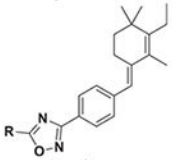
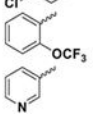
Synthesis of oxadiazole derivatives **(BT-859, BT-860, BT-861, BT-894 and BT-895)**.



Scheme 3.
Synthesis of BT-852, BT-853 and BT-854.

Table 1

Anticancer potential of synthesized compounds in established glioma cell lines and patient derived cell lines (IC_{50} value in μM).

Substituent R=	CODE	D54MG	U251	LN229	DB70	DB93	LogP	CLogP
	BT-851	6.64	19.32	22.78	6.67	3.54	7.22	8.47
	BT-855	100	75	Not tested	Not tested	Not tested	8.05	9.34
	BT-856	>100	>100	Not tested	Not tested	Not tested	5.63	6.97
	BT-892	3.81	6.04	5.93	>50	>50	6.94	8.97
	BT-893	Not tested	20.74	47.50	Not tested	Not tested	7.55	8.83
	BT-904	Not tested	>100	>100	Not tested	Not tested	5.74	6.88
	BT-905	Not tested	4.47	>75	Not tested	Not tested	5.78	7.89
	BT-906	Not tested	Not tested	Not tested	Not tested	Not tested	7.14	8.56
	BT-907	Not tested	>100	>100	Not tested	Not tested	6.37	8.75
	BT-859	>100	7.0	Not tested	Not tested	Not tested	8.39	9.55
	BT-860	>100	10.0	Not tested	Not tested	Not tested	8.12	9.40
	BT-861	65.57	58.36	Not tested	Not tested	Not tested	9.09	9.27
	BT-894	Not tested	37.82	35.59	Not tested	Not tested	6.22	7.28

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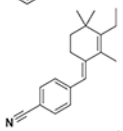
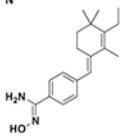
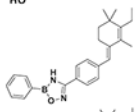
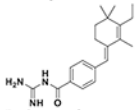
	**BT-895**	Not tested	>100	>100	Not tested	Not tested	6.22	7.28
	**BT-852**	72.21	83.80	Not tested	Not tested	Not tested	5.25	6.57
	**BT-853**	66.61	56.80	Not tested	Not tested	Not tested	4.53	6.02
	**BT-854**	47.40	26.83	60.00	13.34	8.31	6.50	7.68
	**BT-9**	5.60	5.06	6.54	1.77	2.09	3.98	5.41
Postive control	**TMZ**	>500	>500	>1000	25.43	>500	−1.15	−0.81

D54MG- Glioblastoma cell line; U251- Human Glioblastoma cell line; LN229- Human Glioblastoma cell line; DB70- patient derived cell lines; DB93- patient derived cell lines; LogP-Partition coefficient; CLogP- Partition coefficient.