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Synthesis and biological evaluation of novel carbazolyl glyoxamides as anticancer and antibacterial agents

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A new library of 24 carbazolyl glyoxamides **14a-x** were designed and synthesized from glyoxalic acids and arylamines in the presence of HATU as a coupling reagent under MW irradiation. The synthesized carbazolyl glyoxamides were evaluated for their *in vitro* anticancer and antibacterial activities. Of the synthesized carbazolyl glyoxamides, compounds **14l** and **14q** exhibited the most potent cytotoxicity towards breast cancer cell line with IC_{50} values of 9.3 and 9.8 μM, respectively. Further, caspase-3 assay for carbazolyl glyoxamides indicated that these compounds induced apoptotic cell death in Jurkat cells. Furthermore, some of the synthesized carbazolyl glyoxamides **14g**, **14k-l** and **14n** exhibited comparable or even better antibacterial activity (MIC = 8-16 μg/mL) than chloramphenicol against the selected bacterial strains.

Introduction

Indole alkaloids are an important class of natural products and medicinally important molecules, which elicit a wide range of biological activities through diverse mechanisms.[1, 2] Among the indole containing heterocycles, the benzo[*b*]indole system is a carbazole motif that is widely present in a variety of compounds obtained from natural and commercial sources with anticancer and/or antibacterial properties (Fig. 1).[3-6] For example, Fujita *et al* investigated carbazole-based hydrazone, HND-007 (**1**) and related compounds for their *in vivo* antitumor activity suppressing the growth of various cancer cell lines (IC_{50} ~ 1.3–4.6 μM).[7] Pyridine fused carbazole derivative, S16020 (**2**) exhibited potent cytotoxic effects (IC_{50} = 27.5 nM) by virtue of its DNA intercalative and topoisomerase inhibition properties.[8-10] Multidrug resistant tumor cell lines are sensitive to S16020 and it is currently being evaluated in the clinical stages.[11, 12] Naturally occurring carbazole derivative, granulatimide (**3**) was isolated from the ascidian *Didemnum granulatum* and found to display anticancer and antibacterial activities.[13] Caulfield and co-workers had patented carbazolyl chalcone **4** for its potential as a tubulin polymerization inhibitor (IC_{50} = 2 μM).[14] Further, carbazole sulfonamide (**5**), exhibited significant cytotoxicity against leukemia cells (IC_{50} = 19 nM).[15] Nakamura and co-workers reported the isolation of the carbazomycins A-F (**6**) from *Streptoverticillium ehimense* H 1051-MY 10 and these carbazole-based congeners were found to possess promising antibacterial and antifungal activities.[16-18] The current interest in carbazoles for clinical applications arises mainly due to their high efficiencies against several types of diseases, limited toxic side effects, and complete lack of hematological toxicity.[19] In addition to interesting and useful biological applications, carbazole derivatives are also used as organic materials due to their photorefractive, photoconductive, whole transporting and light-emitting properties.[20]

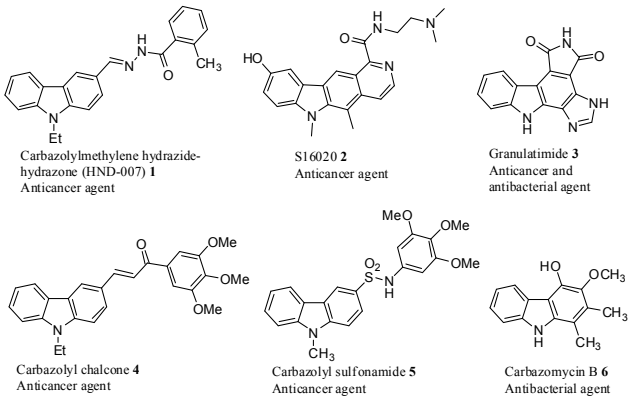

Fig. 1 Carbazole analogues as anticancer and antibacterial agents

Glyoxamide is an important structural unit found in many biologically active compounds and synthetic drug candidates,[21, 22] especially those with anticancer and antibacterial properties. For example, Indibulin (**7**) destabilizes

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tubulin polymerization by arresting tumor cell growth at the G2/M phase. Indibulin is also active in multidrug resistant tumor cell lines and its oral formulation is currently being examined in clinical trials.[23] Structurally similar, conscinamides A-C (**8**) containing an indolic enamide fragment, were isolated from marine sponge *Coscinoderma sp* and found to display antitumor activity against a human prostate cancer cell line (IC_{50} = 7.6 μg/mL)[24] and partial cytoprotection against HIV.[25] More recently, Singh et al. have described the synthesis of different bis(indole)glyoxamides **9** as potent antibacterial candidates.[26] In our continued efforts to find out biologically active indole-based molecules, recently, we have prepared *α*-cyano bis(indolyl)chalcones,[27] 2-arylamino-5-(3′-indolyl)-1,3,4-oxadi-azoles,[28] 5-(3′-indolyl)-1,3,4-thiadiazoles,[29] 2-arylamino-5-(3′-indolyl)-1,3,4-thiadiazoles[30] and indolyl-1,2,4-triazoles[31] as potent anticancer agents. Very recently, we have identified 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides as antibacterial and anticancer agents.[32] Encouraging anticancer and antibacterial activities of glyoxamides and pivotal roles of carbazole scaffold in bioactive compounds prompted us to investigate their new analogues. In this paper, we report a series of 24 carbazolyl glyoxamides **14a–x** by incorporating important scaffolds, glyoxamide and carbazole in a single molecule (Fig. 2).

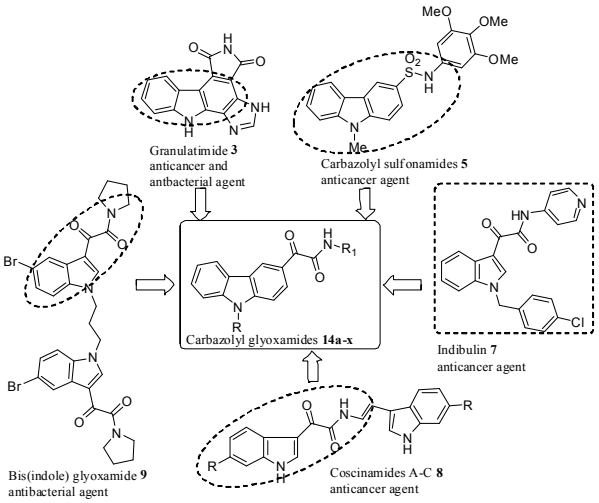
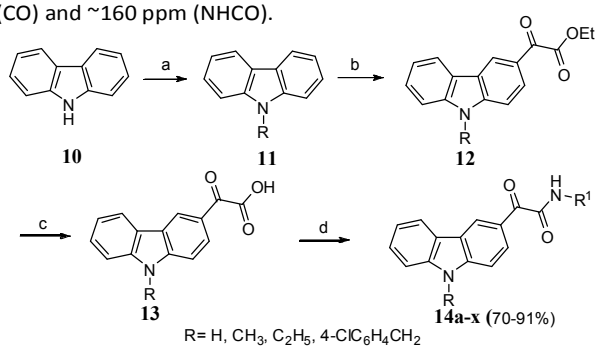


Fig. 2 Design of carbazolyl glyoxamides **14a–x**

Results and discussions

Synthesis and characterization

Carbazolyl glyoxamides **14a–x** were synthesized from the reaction of carbazole **10** with ethyl chlorooxoacetate in the presence of anhydrous $AlCl_3$ followed by ester hydrolysis using LiOH to afford glyoxalic acid **13** in good yield (Scheme 1**)**. For the coupling of **13** with aryl/heteroaryl amines, we optimized the reaction conditions by varying temperatures, solvents, and reagents as illustrated in Table 1. Initial efforts by using thionyl chloride or oxalyl chloride failed to produce **14a** (Table 1, entries 1-2). Subsequent efforts by employing well known carbodiimides including DCC, CDI and EDCI.HCl as coupling reagents under conventional as well as microwave (MW) irradiation conditions generated **14a** in low yield (40%, Table 1. entries 3-6). Finally, the reaction of **13a** and aryl/heteroarylamines in the presence of O-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) at 70 °C under MW irradiation led to **14a** in 70% yield. Scope and generality of this developed protocol was further demonstrated by coupling glyoxalic acid **13** with various arylamines and a series of carbazolyl glyoxamides **14a-x** was prepared in 70-91% yields. Structures of the newly synthesized glyoxamides were well characterized by using spectroscopic techniques including IR, NMR (^1H and ^{13}C) and HRMS. HPLC analysis of synthesized carbazolyl glyoxamides **14a-x** indicated the purity of all the compounds was greater than 97%. In IR spectra of **14a-x**, characteristic peaks at ~1690 and 1655 cm^{-1} were assigned to the carbonyl and amide functionalities, respectively. Further, in ^{13}C NMR spectra of **14a-x**, the carbons of carbonyl and amide functionalities were resonated at ~180 (CO) and ~160 ppm (NHCO).



Scheme 1 Reagents and conditions: (a) CH_3I/C_2H_5I/4-$ClC_6H_4CH_2Cl$, KOH, DMF, rt, 13 h; (b) $Cl(CO)_2OEt$, $AlCl_3$, DCM, 0 °C to rt, 3 h; (c) LiOH, THF:H_2O (1:1), rt, 2 h; (d) R^1NH_2, HATU, DIPEA, DMF, 70 °C, 45 min, MW.

Anticancer activity

Twenty four synthesized glyoxamides were evaluated for their anticancer activities towards human T lymphocyte (Jurkat), histiocytic lymphoma (U937), and breast (MCF-7 and MDA-MB-231) cancer cell lines (Table 2). Structure–activity relationship (SAR) studies of carbazolyl glyoxamides **14a-x** were demonstrated by varying aryl/heteroarylamines and substitution at 9-NH position of carbazoles. Initial screening of compounds **14a-x** at 10 μM concentration indicated that compounds **14i-m** and **14q** displayed about 50% cell survival (Table 2). *N*-Methyl and *N*-ethyl congeners of the carbazole were reported to possess significant cytotoxicity, therefore, derivatives **14r-x** were synthesized.[7, 15] Unfortunately, analogs **14r-x** exhibited low activity against the tested tumor cell lines. The IC_{50} values of selected carbazolyl glyoxamides **14i-m** and **14q** are summarized in Table 3. Compound **14i**, bearing 4-chlorobenzyl and *N,N′*-dimethylaminophenyl moieties was found to show moderate activity. Cytotoxicity was retained by the replacement of a phenyl ring in **14a** with heteroaryl moieties such as 6-quinolyl (**14j**) and 2-(5-methyl)thiazolyl (**14k**).

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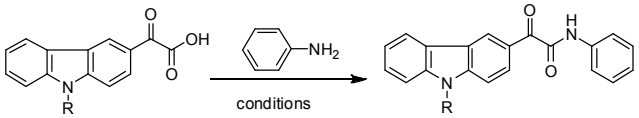
Table 1 Optimization of reaction conditions for the synthesis of carbazolyl glyoxamide **14a**

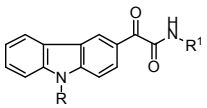


13b **14a** R = 4-ClC$_6$H$_4$CH$_2$

Entry	Reagent	Solvent	Base	Temperature (°C)	Conventional heating		Microwave irradiation	
					Time (h)	Yield (%)	Time (min.)	Yield (%)
1	$SOCl_2$	DCM	TEA	RT	12	Trace	NA	NA
2	$(COCl)_2$	DCM	TEA	RT	15	Trace	NA	NA
3	DCC	THF	TEA	RT	30	Trace	NA	NA
4	CDI	THF	TEA	RT	20	Trace	NA	NA
5	EDCI.HCl/HOBt	THF	DIPEA	RT	14	30	60	40
6	EDCI.HCl/HOBt	DMF	TEA	70	12	Trace	60	Trace
7	HATU	THF	DIPEA	60	15	Trace	60	Trace
8	HATU	DMF	DIPEA	70	12	40	45	70
9	HATU	DMF	TEA	70	12	20	45	40

RT: room temperature; NA: not attempted

Table 2 *In vitro* cytotoxicity of carbazolyl glyoxamides **14a-x**



			Percentage cell survival (@ 10 μM)			
Compound	R	R^1	Jurkat	U937	MCF-7	MDA-MB-231
14a	4-ClC$_6$H$_4$CH$_2$	C$_6$H$_5$	71.5±10.7	88.5±5.2	69.5±7.8	82.3±7.5
14b	4-ClC$_6$H$_4$CH$_2$	4-CH$_3$C$_6$H$_4$	70.2±0.8	79.5±3.4	67.1±7.4	83.5±6.8
14c	4-ClC$_6$H$_4$CH$_2$	4-CH$_3$OC$_6$H$_4$	71.3±26.7	82.1±2.2	74.6±0.9	80.5±5.5
14d	4-ClC$_6$H$_4$CH$_2$	3-CH$_3$OC$_6$H$_4$	71.3±14.4	67.2±8.5	70.9±10.4	78.6±6.8
14e	4-ClC$_6$H$_4$CH$_2$	3,4-(CH$_3$O)$_2$C$_6$H$_3$	69.8±14.7	85.5±5.3	67.3±4.6	72.5±2.2
14f	4-ClC$_6$H$_4$CH$_2$	3,4,5-(CH$_3$O)$_3$C$_6$H$_2$	64.5±1.4	98.8±2.9	73.7±19.9	70.9±5.2
14g	4-ClC$_6$H$_4$CH$_2$	4-FC$_6$H$_4$	118.4±26.4	83.2±6.7	93.3±5.6	88.9±6.4
14h	4-ClC$_6$H$_4$CH$_2$	4-pyridyl	83.1±19.6	59.1±14.8	66.3±5.4	90.2±2.2
14i	4-ClC$_6$H$_4$CH$_2$	4- (CH$_3$)$_2$*N,N*-C$_6$H$_4$	**56.5±6.9**	78.8±1.2	70.5±5.8	**62.8±3.5**
14j	4-ClC$_6$H$_4$CH$_2$	6-quinolyl	**56.5±1.6**	**60.3±17.4**	68.0±7.0	**63.5±2.9**
14k	4-ClC$_6$H$_4$CH$_2$	2-(5-methyl)thiazolyl	**51.3±0.5**	**62.2±5.8**	58.4±6.6	**64.8±1.9**
14l	H	C$_6$H$_5$	83.4±41.0	**64.4±8.7**	**48.5±4.0**	75.2±2.4
14m	H	3,4,5-(CH$_3$O)$_3$C$_6$H$_2$	**52.4±5.9**	67.4±5.8	**59.1±13.3**	61.3±2.0
14n	H	3-CH$_3$OC$_6$H$_4$	60.7±0.7	72.1±6.9	59.8±19.9	72.6±8.3
14o	H	4-CH$_3$OC$_6$H$_4$	77.9±9.4	85.6±6.5	89.4±17.3	84.9±10.5
14p	H	4-pyridyl	94.9±6.3	92.8±5.2	60.5±10.6	98.2±5.4
14q	H	3,4-(CH$_3$O)$_2$C$_6$H$_3$	60.5±6.2	65.8±8.5	**50.0±7.3**	65.2±4.5
14r	CH$_3$	C$_6$H$_5$	95.31±7.2	109.7±10.4	104.2±6.7	94.7±6.2
14s	CH$_3$	3,4,5-(CH$_3$O)$_3$C$_6$H$_2$	99.77±6.6	107.5±9.3	72.1±5.4	92.3±3.9
14t	C$_2$H$_5$	C$_6$H$_5$	93.7±5.3	91.3±10.9	114.2±11.3	94.5±5.2
14u	C$_2$H$_5$	3,4-(CH$_3$O)$_2$C$_6$H$_3$	92.3±2.2	104.7±9.5	106.9±12.1	96.5±6.6
14v	C$_2$H$_5$	3,4,5-(CH$_3$O)$_3$C$_6$H$_2$	100.4±10.8	106.9±7.4	78.1±8.8	88.8±7.1
14w	C$_2$H$_5$	4- (CH$_3$)$_2$*N,N*-C$_6$H$_4$	102.3±9.7	93.6±4.2	79.7±7.6	89.9±9.2
14x	C$_2$H$_5$	6-quinolyl	100.4±10.8	95.7±7.2	73.8±8.5	83.9±8.9
Control (negative)			100±5.9	101.5±9.5	100±3.2	100±2.2
Doxorubicin (positive)			**21.2±5.6**	**31.8±1.1**	**38.2±10.6**	**40.2±3.5**

The activity data represent mean values ± SD of experiments conducted in triplicates at three independent times

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Table 3 IC_{50} values (μM) of selected carbazolyl glyoxamides

S. No	Jurkat	U937	MCF-7	MDA-MB-231
14i	10.5 ± 2.1	29.2 ± 3.9	23.5 ± 8.2	17.9 ± 5.7
14j	11.3 ± 3.6	15.1 ± 4.8	18.9 ± 3.8	18.7 ± 7.7
14k	10.2 ± 2.9	17.5 ± 8.2	12.2 ± 5.4	20.1 ± 8.4
14l	11.8 ± 3.3	18.3 ± 7.8	**9.3 ± 4.3**	31.2 ± 11.4
14m	12.1 ± 1.8	23.3 ± 10	11.5 ± 5.5	14.7 ± 5.4
14q	17.8 ± 3.2	29.2 ± 9.2	**9.8 ± 2.8**	18.5 ± 5.4
DX-1	0.25 ± .11	0.15 ± .05	0.35 ± .15	0.5 ± .20

*Bold value indicates IC_{50} > 10 μM; DX-1 = Doxorubicin

Compound **14l** with *N*-H free carbazole and *N*-phenyl glyoxamide was identified as the most active member of the series with IC_{50} values between 9.3 to 31.2 μM. Also, compound **14l** was found to be 2-3 fold more cytotoxic towards MCF-7 (IC_{50} = 9.3 μM) and Jurkat (IC_{50} = 11.8 μM) cells. No significant change in activity was observed by the replacement of a phenyl group in compound **14l** with a trimethoxyphenyl (**14m**) and dimethoxyphenyl (**14q**) groups except for **14q** (IC_{50} = 9.8 μM; MCF-7). However, compound **14q** was found to be equipotent (compounds **14l** vs **14q**) against MCF-7 cell line (IC_{50} = 9.8 μM). From the structural variation it was realized that carbazole-*N*-H with its appended glyoxamide bearing phenyl, dimethoxyphenyl and trimethoxyphenyl units increases cytotoxicity.

To further characterize the mode of cellular death by carbazolyl glyoxamides, apoptosis induction studies for the selected compounds **14i**-**m** and **14q** were performed on Jurkat cells by the caspases 3/7 activation method. Caspases belonging to a family of cysteine proteases are known to play an essential role in apoptosis.[33] Out of these caspases, caspase-3 is an effector caspase that cleaves multiple proteins in cells leading to apoptotic cell death. Therefore, activation of caspase 3 pathway is a hallmark of apoptosis and can be used in cellular assay to quantify activator. Of the carbazolyl glyoxamides tested, compounds **14i**, **14k**, **14l** and **14q** showed 4-5-fold enhancement in caspase level compared to the control (Fig 3). These results imply that carbazolyl glyoxamides induced apoptosis in Jurkat cells *via* caspase-3-dependent pathway.

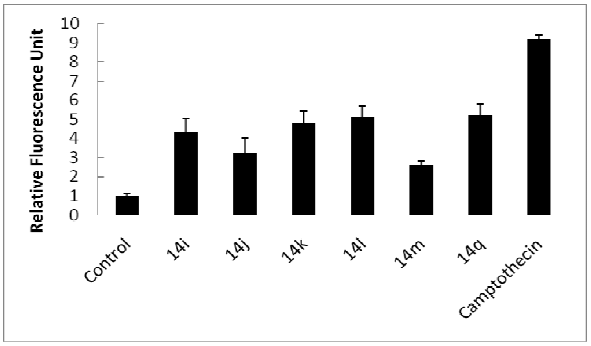

Fig. 3 Carbazolyl glyoxamides **14i-m** and **14q** induced caspase activation in Jurkat cells

Antibacterial activity

In light of interesting antibacterial activities of many carbazole containing natural and synthetic compounds, the newly synthesized carbazolyl glyoxamides **14a-x** were screened for their antibacterial activity.[27] All the compounds were tested for their *in vitro* antibacterial activities against Gram-positive bacteria including *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121), and Gram-negative bacteria including *Escherichia coli* (MTCC 1652) and *Pseudomonas putida* (MTCC102) with respect to chloramphenicol, a standard drug. The Minimum Inhibitory Concentrations (MICs) and Zone of Inhibition (ZOI) for compounds **14a-x** were determined *in vitro* by the modified broth micro-dilution values method as given in Table 4. Compounds containing *N*-chlorobenzyl carbazole and C_6H_5 (**14a**), $CH_3C_6H_4$ (**14b**) $CH_3OC_6H_4$ (**14c**), $(CH_3O)_3C_6H_2$ (**14f**) and *N,N'*-$(CH_3)_2C_6H_4$ (**14i**) substituents in the glyoxamide fragment were found to display moderate activity against the tested bacterial strains. Interestingly, introduction of an electron-withdrawing fluoro group in the phenyl ring resulted in **14g** endowed with potent antibacterial activity against all tested bacterial strains with MIC values ranging between 8 to16 μg/mL. Replacement of a *N*-phenyl ring in **14a** with heteroaryl groups such as 4-pyridyl (**14h**) and 6-quinolyl (**14j**) led to inactive derivatives; except **14k** bearing 2-(5-methyl)thiazolyl) moiety exhibited comparable antibacterial activity against *B. cereus* and *E. coli* bacterial strains (ZOI = 16-19 mm; MIC = 16 μg/mL). Compound **14l** with carbazole *N*-H and a phenyl moiety on the amide part displayed improved activity when compared to the corresponding *N*-substituted carbazole **14a**. Replacement of a phenyl ring in **14l** by methoxyphenyl (**14m-o** and **14q**) or 4-pyridyl (**14p**) moiety led to a decrease in the activity except for **14n** (MIC = 8-16 μg/mL). Alkylations of carbazole *N*-H resulted in compounds **14r-x** with moderate activity against the tested bacterial strains.

Cell viability assay

Although antibacterial (ZOI & MIC) assays might show a potential to kill pathogenic micro-organisms, concentration vs time curves provide more insight about the rate of antibacterial activity. To determine the rate of bactericidal activity of the two most active compounds **14g** and **14l**, time-kill studies were performed. The interactive time and the change in the number of microorganisms for compounds **14g** and **14l** is presented in Fig. 4A-D.

The percent population reduction at different time interval was calculated to demonstrate the change of the population of microorganism respect to concentration dependent dose. The compound **14g** showed high bacteriostatic effect against *E. coli* in the first hours of incubation.

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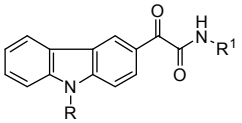
Table 4 *In vitro* antibacterial activities of carbazolyl glyoxamides **14a-x**



Compound	R	R^1	Gram-positive bacteria				Gram-negative bacteria			
			B. cereus		*S. aureus*		*E. coli*		*P.putida*	
			ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
14a	4-$ClC_6H_4CH_2$	C_6H_5	16	32	16	>32	14	64	15	32
14b	4-$ClC_6H_4CH_2$	4-$CH_3C_6H_4$	15	>32	-	-	16	64	-	-
14c	4-$ClC_6H_4CH_2$	4-$CH_3OC_6H_4$	16	32	15	32	17	>16	15	>16
14d	4-$ClC_6H_4CH_2$	3-$CH_3OC_6H_4$	17	16	-	-	14	>16	-	-
14e	4-$ClC_6H_4CH_2$	3,4-$(CH_3O)_2C_6H_3$	15	>64	17	64	15	32	16	>16
14f	4-$ClC_6H_4CH_2$	3,4,5-$(CH_3O)_3C_6H_2$	15	>32	17	>64	18	32	16	32
14g	4-$ClC_6H_4CH_2$	4-FC_6H_4	**18**	**16**	**18**	**>8**	**20**	**8**	**19**	**16**
14h	4-$ClC_6H_4CH_2$	4-pyridyl	15	128	17	>32	16	32	16	32
14i	4-$ClC_6H_4CH_2$	4-$(CH_3)_2N$,*N*-C_6H_4	16	64	18	>32	14	32	16	64
14j	4-$ClC_6H_4CH_2$	6-quinolyl	15	>32	16	>128	15	32	18	64
14k	4-$ClC_6H_4CH_2$	2-(5-methyl)thiazolyl	16	**16**	**19**	**>8**	**19**	**16**	**18**	>16
14l	H	C_6H_5	**17**	**16**	**18**	**>8**	**19**	>8	**17**	**8**
14m	H	3,4,5-$(CH_3O)_3C_6H_2$	15	>16	17	>16	16	32	16	64
14n	H	3-$CH_3OC_6H_4$	**18**	**>8**	**19**	**16**	16	**16**	**18**	**8**
14o	H	4-$CH_3OC_6H_4$	14	64	14	>64	17	128	15	>64
14p	H	4-pyridyl	15	32	16	>64	18	32	16	>32
14q	H	3,4-$(CH_3O)_2C_6H_3$	16	128	18	>32	17	>16	14	>64
14r	CH_3	C_6H_5	13	>32	13	64	15	32	14	32
14s	CH_3	3,4,5-$(CH_3O)_3C_6H_2$	14	64	13	32	17	32	15	>32
14t	C_2H_5	C_6H_5	15	>32	14	64	16	>16	17	>16
14u	C_2H_5	3,4-$(CH_3O)_2C_6H_3$	14	64	-	-	16	>32	16	>16
14v	C_2H_5	3,4,5-$(CH_3O)_3C_6H_2$	15	>16	15	>32	14	>64	-	-
14w	C_2H_5	4- $(CH_3)_2N$,*N*-C_6H_4	15	>32	14	>64	16	32	16	32
14x	C_2H_5	6-quinolyl	12	32	14	32	13	>32	14	64
Chloramphenicol			21	32	21	16	22	16	21	16

*ZOI (in mm) and MIC (in µg/mL) values, bold values indicate comparable or even better antibacterial activity than chloramphenicol.

Moreover, the highest reduction (85%) in *E. coli* population was observed at 8 h of incubation with compound **14g** (Fig. 4A). Compound **14g** was also showed about >70% reduction in viable cells of *S. aureus* (Fig. 4B). Compound **14l** with free carbazole *N*-H and a phenyl moiety in amidic part led to significant inhibition of bacterial growth after 4 h of incubation. It is evident that within 8 h, compound **14l** exhibited almost 80% reduction in the viability of *P. Putida* (Fig. 4C). Similarly, bacterostatic effect of compound **14l** against *S. aureus* reveals about 70% reduction of viable cell at 8 h of incubation (Fig. 4D). Thus the results of present study revealed that **14g** and **14l** were capable of inhibiting the bacterial growth within few hours of initial interactions.

The toxicity of potent compounds **14i-m** and **14q** was evaluated using LDH (Lactate dehydrogenase) assay. The LDH activity shows that all the tested compounds **14i-m** and **14q** exhibited lower toxicity than the standard drug, doxorubicin which justifies the potential use of these compounds as anti-bacterial agents (Fig. 5).

From the structure-activity relationship (SAR) studies of carbazolyl glyoxamides it implies that a combination of carbazole *N*-H and glyoxamide unit possessing *p*-fluorophenyl and methoxyphenyl substituents are beneficial for the activity (Fig. 6). All the synthesized compounds demonstrated well to moderate cytotoxicity against a panel of cancer cell lines and excellent to moderate antibacterial activity towards tested bacterial strains. Particularly, compound **14l** with carbazole *N*-H and phenyl moietiy in glyoxamide part exhibited potent cytotoxicity and antibacterial activities. Exclusively, with carbazole *N*-H and dimethoxyphenyl moieties, compound **14q** was found to be the most active against the tested cancer cell lines and less potent towards tested bacterial strains. However, compound **14g** with *N*-chlorobenzylcarbazole and fluorophenyl units, and analogue **14n** having carbazole *N*-H and 3-methoxyphenyl moieties, were the most active carbazolyl glyoxamides against the tested bacteria but exhibited low cytotoxicity towards tested cancer cell lines.

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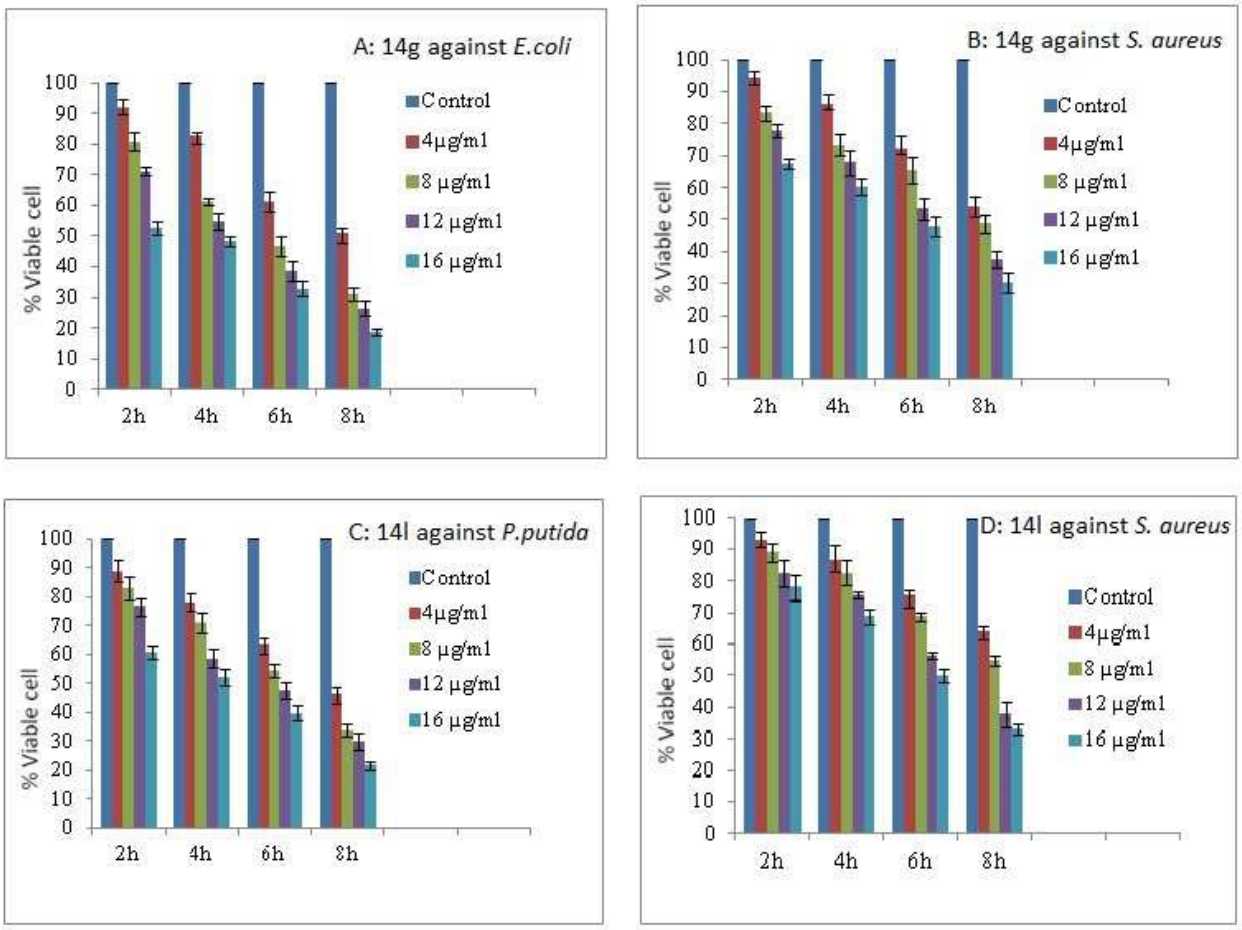

Fig. 4 Cell viability assay of **14g** and **14l** against selected bacteria

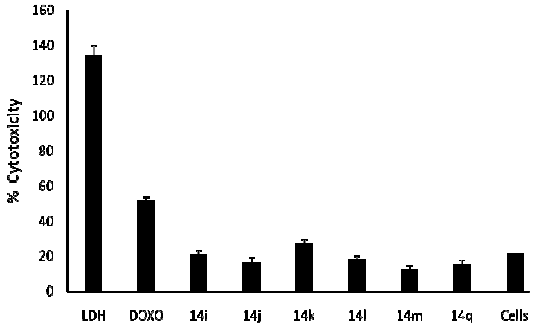

Fig. 5 Cytoxicity induced by carbazolyl glyoxamides in terms of LDH release

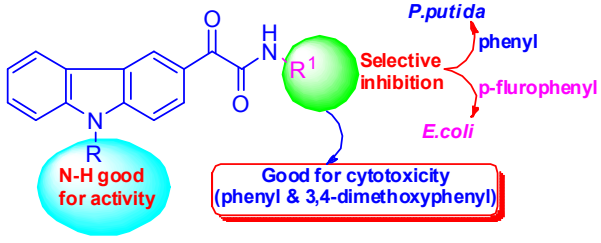

Fig. 6 Structure-activity relationship of carbazolyl glyoxamides **14a-x**

Conclusions

In summary, we have synthesized various carbazolyl glyoxamides from readily available glyoxalic acids **13** and arylamines by employing HATU as a coupling reagent. Synthesized glyoxamides were assessed for their cytotoxicity which enabled us to identify **14l** and **14q** as the most potent compounds against MCF-7 cells with IC_{50} values of 9.3 µM and 9.8 µM, respectively. Preliminary mechanism of action studies indicated that carbazolyl glyoxamides induced apoptosis in Jurkat cells *via* caspase-3 and-7 activation. In addition, antibacterial activity evaluation led us to compounds **14g, 14k-l** and **14n** with significant potency against Gram-positive and Gram-negative bacteria (MIC = 8-16 µg/mL and ZOI = 16-20 mm). Antibacterial activities of potent compounds **14g**, **14k-l** and **14n** were found to be comparable to the reference drug, chloramphenicol. Cell viability assay revealed that analogues **14g** and **14l** were capable of inhibiting the bacterial growth within few hours of initial interactions. Interesting activity results indicate that the identified potent carbazolyl glyoxamides **14g** and **14l** can be exploited further to develop either highly specific or potent antibacterial/ anticancer

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agents, or if required both of these properties could be incorporated in the same molecule.

Experimental

General procedure for the synthesis of carbazolyl glyoxamides (14a-x).

To a 10 mL microwave tube was added carbazole glyoxalic acid 13 (0.275 mmol), HATU (0.12 g, 0.317 mmol), N,N-diisopropylethyl-amine (0.09 g, 0.687 mmol) and an appropriate aryl/heteroarylamine (0.303 mmol) in DMF (2 mL). The tube was sealed with a pressure cap and placed in the microwave cavity. The sample was irradiated for 45 min at 70 °C and then allowed to cool at room temperature. The residue was poured into ice-cold water (30 mL) and stirred for 20 min at room temperature. The solid so obtained was filtered, dried and purified by column chromatography on silica gel using ethylacetate:hexane (3:7) as eluent to give pure **14a-g**, **14j-o** and **14q-x** in excellent yields. Some of the compounds (**14h-i** and **14p**) were crystallized from acetone to obtain pure products in 70-91% yields.

2-(9-(4-Chlorobenzyl)-9H-carbazol-3-yl)-2-oxo-N-phenyl-acetamide (14a).

Yellow solid; Yield 70%; M.p: 191-193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (d, J = 1.3 Hz, 1H), 9.15 (s, 1H), 8.58 (dd, J = 8.8, 1.7 Hz, 1H), 8.25 (dd, J = 7.6, 1.1 Hz, 1H), 7.78 (dd, J = 8.6, 1.0 Hz, 2H), 7.54-7.50 (m, 1H), 7.45 (dd, J = 8.3, 7.5 Hz, 3H), 7.41–7.36 (m, 3H), 7.26 (t, J = 2.6 Hz, 2H), 7.08 (d, J = 8.6 Hz, 2H), 5.55 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.9, 160.0, 144.2, 141.2, 136.9, 134.6, 133.7, 129.8, 129.3, 129.2, 127.7, 127.0, 126.2, 125.2, 125.0, 123.6, 123.1, 121.1, 121.1, 120.0, 109.5, 108.8, 46.24; IR (KBr, v, cm^{-1}): 3335, 3090, 3052, 2916, 1682, 1653, 1589, 1520, 1435, 1307, 1250, 1134, 1011, 825, 795; Anal. RP-HPLC t_R = 4.641 min, purity 98.55%; HRMS (ESI$^+$) calculated for $C_{27}H_{20}ClN_2O_2$ $[M+H]^+$, 439.1213; Found 439.1208 and 461.1025 $[M+Na]^+$.

2-(9-(4-Chlorobenzyl)-9H-carbazol-3-yl)-2-oxo-N-(p-tolyl) acetamide (14b).

Yellow solid; Yield 72%; M.p: 172-173 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.48 (d, J = 1.6 Hz, 1H), 9.09 (s, 1H), 8.58 (dd, J = 8.8, 1.7 Hz, 1H), 8.27–8.23 (m, 1H), 7.67–7.64 (m, 2H), 7.53-7.49 (m, 1H), 7.44–7.35 (m, 3H), 7.26 (t, J = 2.2 Hz, 3H), 7.24 (s, 1H), 7.09 (d, J = 8.6 Hz, 2H), 5.55 (s, 2H), 2.39 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.0, 159.9, 144.2, 141.1, 134.9, 134.6, 134.3, 133.7, 129.9, 129.7, 129.2, 127.7, 126.9, 126.1, 125.1, 123.6, 123.1, 121.1, 121.0, 119.9, 109.5, 108.7, 46.2, 21.0; IR (KBr, v, cm^{-1}): 3325, 3094, 3055, 2916, 1682, 1651, 1620, 1582, 1520, 1443, 1327, 1265, 1149; Anal. RP-HPLC t_R = 5.317 min, purity 98.10%; HRMS (ESI$^+$) calculated for $C_{28}H_{21}ClN_2O_2$ $[M+H]^+$, 453.1369; Found 453.1365 and 475.1183 $[M+Na]^+$.

2-(9-(4-Chlorobenzyl)-9H-carbazol-3-yl)-N-(4-methoxyphenyl) -2-oxoacetamide (14c).

Yellow solid; Yield 72%; M.p: 168-170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (d, J = 1.5 Hz, 1H), 9.04 (s, 1H), 8.56 (dd, J = 8.8, 1.7 Hz, 1H), 8.22 (d, J = 7.7 Hz, 1H), 7.69–7.65 (m, 2H), 7.51-7.47 (m, 1H), 7.42–7.32 (m, 4H), 7.25–7.23 (m, 1H), 7.06 (d, J = 8.5 Hz, 2H), 6.97–6.94 (m, 2H), 5.52 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.1, 159.8, 157.0, 144.2, 141.1, 134.6, 133.7, 130.1, 129.7, 129.2, 127.7, 126.9, 126.1, 125.1, 123.6, 123.1, 121.6, 121.1, 121.0, 114.4, 109.5, 108.7, 55.5, 46.2; IR (KBr, v, cm^{-1}): 3348, 3055, 2924, 1682, 1643, 1620, 1582, 1528, 1443, 1327, 1250, 1149; Anal. RP-HPLC t_R = 4.368 min, purity 98.67%; HRMS (ESI$^+$) calculated for $C_{28}H_{22}ClN_2O_3$ $[M + H]^+$, 469.1319; Found 469.1310 and 491.1129 $[M+Na]^+$.

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