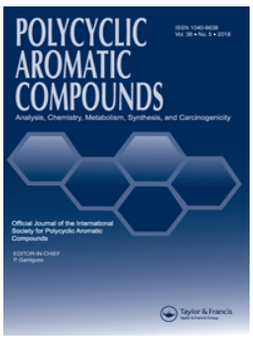

Polycyclic Aromatic Compounds

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Microwave Irradiated Targeted Synthesis of Pyrrolobenzodiazepine Embrace 1,2,3-Triazole by Click Chemistry Synthetic Aspect and Evaluation of Anticancer and Antimicrobial Activity

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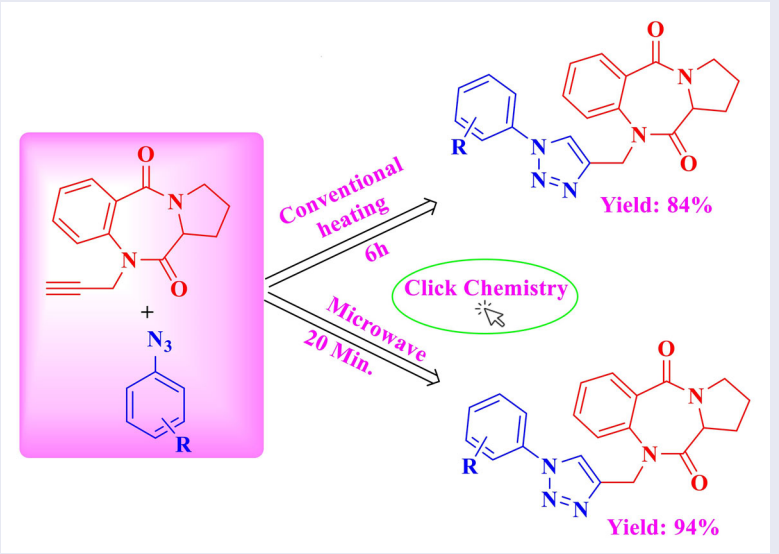
ABSTRACT

A new series of pyrrolobenzodiazepine derivatives containing 1,2,3-triazoles moiety has been designed and developed via Cu(I)-catalyzed azide-alkyne cycloaddition reaction, click chemistry synthetic aspect under microwave irradiation. The reaction has been curtained in diverse azide derivatives, solvent ratio, and catalyst for different time duration. The reaction has been studied with different azide substrate scope and proposed reaction mechanism has also been projected. Synthesized compounds also screening for *in vitro* anticancer activity and antimicrobial activity.



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Introduction

In current scenario grouping of two different pharmacophores with distinct or similar mode of accomplishment to get synthesis of significant final pharmacophore. Benzodiazepines comprise a significant class of bioactive molecules and their combination has been getting much consideration in the field of medicinal and pharmaceutical chemistry in arrears to their application such as anti-inflammatory, anti-analgesic, anticonvulsant, and hypnotic.[1–4] The first drug containing pyrrolo[2,1-*c*][1,4]benzodiazepine (**PBD**) moiety was anthramycin in 1960s in culture of *Streptomyces*.[5] Pyrrolo[2,1-*c*][1,4]benzodiazepine (**PBD**) antibiotics are a family of natural products by diverse *actinomycetes* bacteria, and they are also known for their antitumor and antibiotic application.[6–8] Some naturally occurring pyrrolo[2,1-*c*][1,4]benzodiazepine show in Figure 1. These PBD compounds shows anticancer activity because of their covalent obligatory to the C_2-NH_2 guanine base group.[9] Since the disclosure of natural PBDs designed, prepared, and discovered by different synthetic approach alike monomers, dimers, and hybrids.[10,11] A synthetic dimer of PBD, SJG-136 has endured clinical estimation as a monotherapy[12] (Figure 2). PBD monomers length three DNA base sets with an inclination for 5′-Pu-G-Pu, especially 5′-AG or 5′-GA sequences.[13]

Furthermore, another heterocyclic moiety 1,2,3-triazoles which are prepared from regioselective synthesis of terminal alkynes and azides under Cu(I)-catalyzed azide-alkyne cycloaddition **(CuAAC)** have all been quantified as a click chemistry.[14–20] Compounds containing 1,2,3-triazole ring parade significant biological response alike antimicrobial, antiviral, anticancer,[21,22] and anti-inflammatory. Which has been discovered various application in bioconjugate science. They have been described to be inhibitors of glycogen synthase kinase-3,[23] agonists of muscarine receptors,[24] agonists of GABA receptors.[25,26]

On the basis of aforementioned literature and extension of our studies on the development of new triazole containing pyrrolobenzodiazepine derivatives. Our efforts on synthesize more active

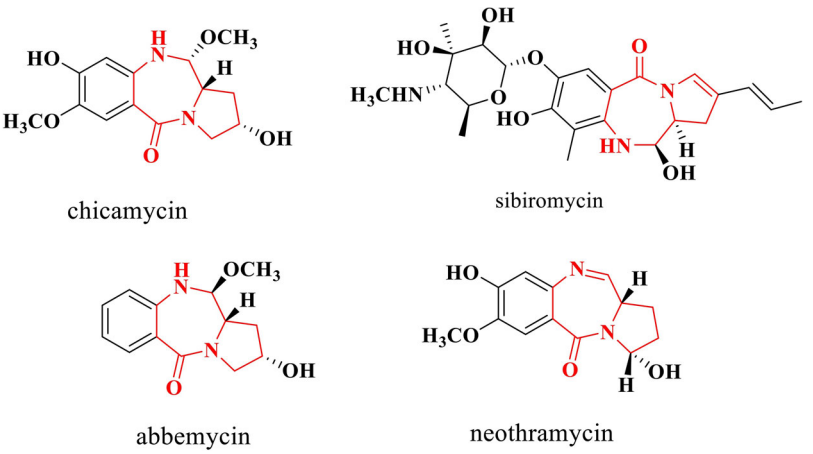


Figure 1. Some naturally occurring PBDs.

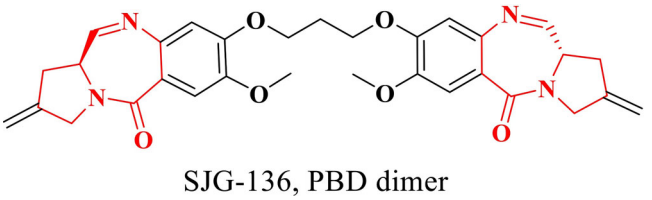


Figure 2. Structure of PBD dimer.

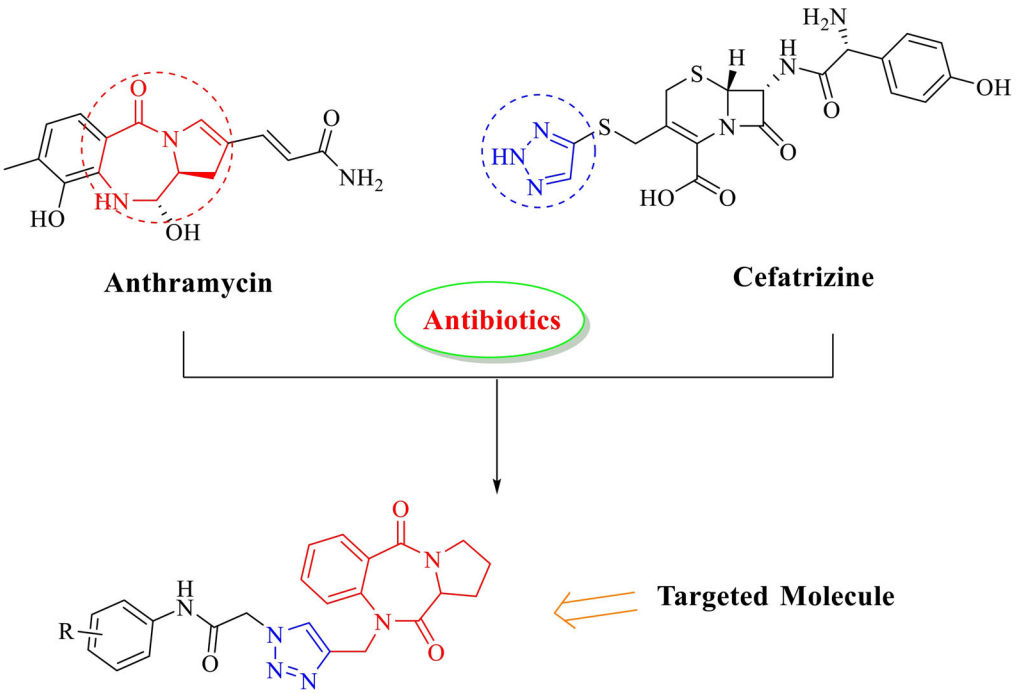


Figure 3. Grouping of two pharmacophores in targeted molecule.

pharmacological molecules which are combination of different pharmacophore with similar activities. pyrrolobenzodiazepine (**PBD**) and 1,2,3-triazole which moiety containing in recent marketed antibiotics as well as antitumor drug like anthramycin and cefatrizine, so, our targeted molecule is grouping of both pharmacological core in one molecule, which is shown more biological response (Figure 3).

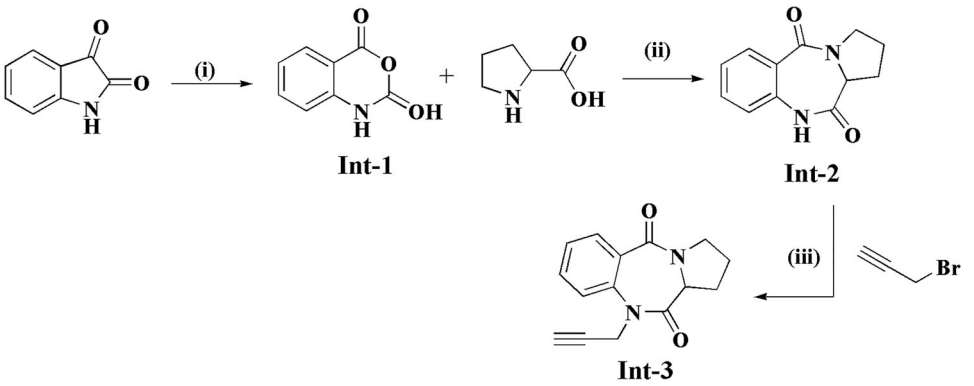
Results and discussion

Chemistry

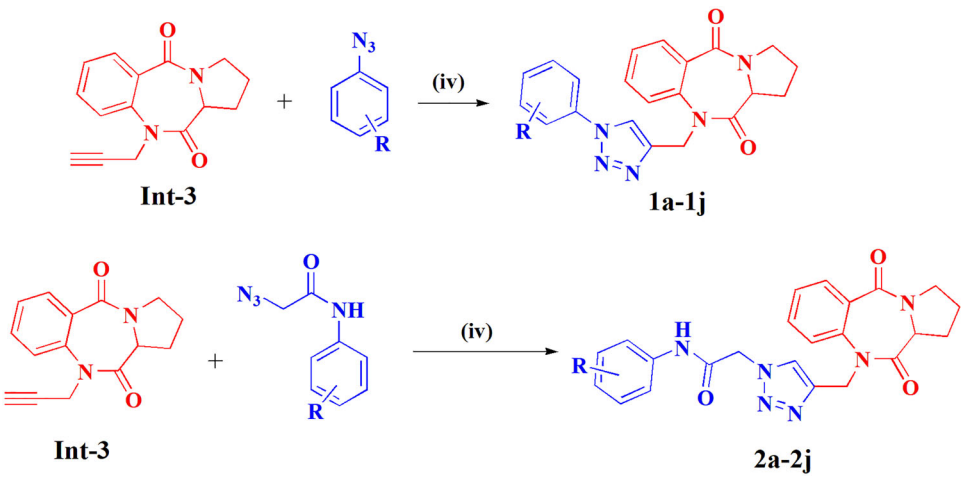
In prolongation of our previous efforts toward the synthesize of various biologically active heterocyclic scaffolds by microwave irradiation.[27,28] We report herein novel pyrrolobenzodiazepine derivatives containing 1,2,3-triazole moiety under carried out microwave irradiation.

The synthesis of pyrrolobenzodiazepine derivatives derived from isatin as starting material further it reacts and gives isatoic anhydride (**Int-2**) after that addition of proline gives PBD (**Int-2**). For click chemistry Cu(I)-catalyzed azide-alkyne cycloaddition **(CuAAC)** reaction is required so, PBD reacts with propargyl bromide to generate terminal alkyne position (**Int-3**) (Scheme 1). It further reacts with substituted phenyl azide to obtained desired adducts (Scheme 2).

The reaction is screened under various solvent like MeOH, DMF, Water, BuOH, *t*-BuOH, condition, and various catalyst combination of Na-ascorbate and $CuSO_4$, CuI (10%), CuBr (10%) for different time by various methods like conventional method and microwave irradiation method at room temperature to get different yields. The optimization of reaction conditions for synthesized compound **1a** is summed up in Table 1. Comparison between conventional heating method and microwave irradiation method among the microwave method is rapid and efficient compare to conventional method. We were tired DMF:*t*-BuOH:Water (1:1:1) solvent system in conventional method in presence of Cu and Na-ascorbate catalyst at 70 °C temperature for 6 h we got a 84% isolated yield obtained but same reaction we done by microwave assisted method at 60 °C



Scheme 1. Preparation of PBD contain tertiary alkyne position. Reagents and condition (i). m-CPBA, THF, 0–5 °C to R.T. for 4 h, (ii). DMSO, 120 °C, 3 h, (iii). K_2CO_3, Acetone 6 h.



Scheme 2. Series of Click chemistry on PBDs with substituted azides. Reagents and condition (iv). $CuSO_4$, Na-ascorbate, DMSO:H_2O:*tert*-butanol, M.W. for 30–35 min.

Table 1. Optimization of reaction conditions of compound **1a**.

Entry	Solvent	Catalyst	Time (h)	**Yield**[a]
1	MeOH	$CuSO_4$, Na-ascorbate	12	37[b]
2	DMF:MeOH(1:1)	$CuSO_4$, Na-ascorbate	12	52[b]
3	DMF:t-BuOH(1:1)	$CuSO_4$, Na-ascorbate	8	65[b]
4	**DMF:*t*-BuOH:Water(1:1:1)**	**$CuSO_4$, Na-ascorbate**	**6**	**84**[b]
5	DMF:Water(1:1)	$CuSO_4$, Na-ascorbate	8	62[b]
6	BuOH:Water(1:1)	$CuSO_4$, Na-ascorbate	8	72[b]
7	Water	$CuSO_4$, Na-ascorbate	12	18[b]
8	***DMF:t-BuOH:Water(1:1:1)***	***$CuSO_4$, Na-ascorbate***	***20 min.***	***94***[c]
9	DMF:t-BuOH:Water(1:1:1)	CuI (10%)	20 min.	75[c]
10	DMF:t-BuOH:Water(1:1:1)	CuBr (10%)	30 min.	67[c]

Bold and italic values indicate final optimal conditions.
[a]Isolated yield in percentage.
[b]Conventional heating method at 70 °C temp.
[c]Microwave irradiation method at 60 °C temp and 180 W.

temperature and 180 W for 20 min we got 94% isolated yield obtained. So, comparatively microwave method is more efficient, less time consuming and yield increment method comparative to conventional heating method (Figure 4).

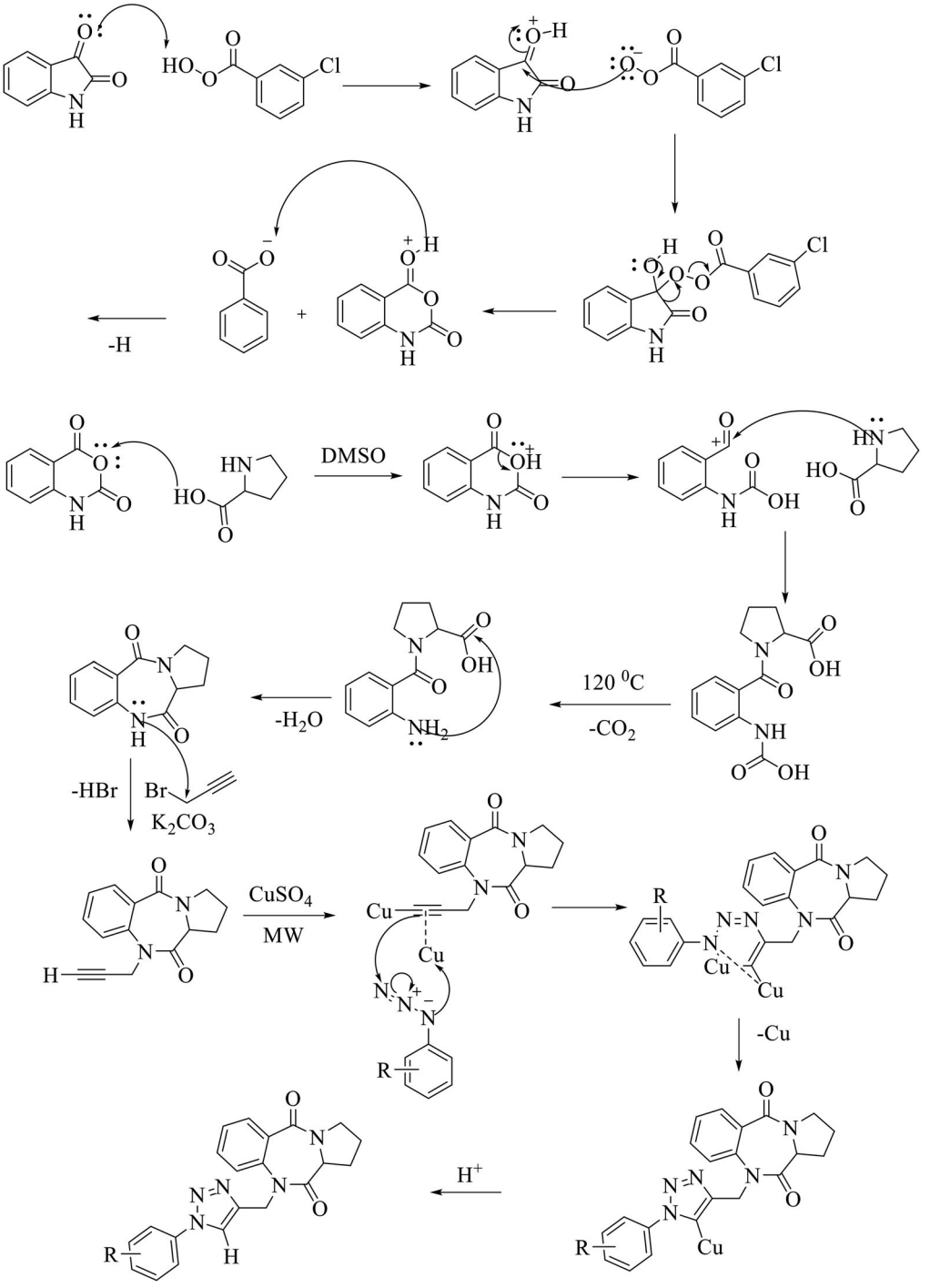


Figure 4. Proposed reaction mechanism.

Table 2. Anticancer screening data.

Compound name	Cell line/Cancer type	Growth Percent	% growth inhibition
1a	EKVX/ nscL	87.46	12.54
	UO-31/ RC	80.76	19.24
1b	SNB-75/ CNS	90.15	9.85
	UO-31/ RC	82.91	17.09
1c	SNB-75/ CNS	56.55	43.45
	CAKI-1/ RC	87.76	12.24
	UO-31/ RC	84.06	15.94
1d	SNB-75/ CNS	81.33	18.67
	UO-31/ RC	73.33	26.67
1i	NCI-H226/ nscL	87.08	12.92
	SNB-75/ CNS	81.68	18.32
	UO-31/ RC	78.89	21.11
2h	EKVX/ nscL	89.52	10.48
	NCI-H522/ nscL	87.64	12.36
	UO-31/ RC	83.58	16.42
2j	NCI-H226/ nscL	79.86	20.14
	SF-268/ CNS	89.55	10.45
	SNB-75/ CNS	73.4	26.6
	SK-OV-3/ OC	80.88	19.12
	CAKI-1/ RC	87.92	12.08
	UO-31/ RC	80.42	19.58
	MCF7/ BC	84.12	15.88
	HS 578T/ BC	76.58	23.42
	T-47D/ BC	80.56	19.44

L: Leukemia; nscL: Non-Small Cell Lung Cancer; CNS: CNS Cancer; OC: Ovarian Cancer; RC: Renal Cancer; BC: Breast Cancer.

Table 3. Secondary antimicrobial screening of synthesized compounds[a].

		Zones of growth inhibition[b]			
		Gram-positive bacteria		Gram-negative bacteria	
Entry	Comp. code	*Bacillus megaterium*	*Bacillus subtilisis*	*E. coli*	*Klebsiella*
1	1a	9	8	10	9
2	**1b**	**7**	**5**	**15**	**16**
3	**1c**	**14**	**13**	**7**	**11**
4	1d	9	6	12	9
5	1e	4	5	6	9
6	1f	0	nt	4	5
7	**1g**	**16**	**13**	**7**	**12**
8	1h	3	0	10	10
9	**1i**	**10**	**9**	**12**	**15**
10	1j	8	4	6	9
11	2a	11	8	6	7
12	2b	6	5	10	9
13	2c	7	6	8	5
14	2d	7	2	9	6
15	2e	10	8	3	7
16	2f	9	8	4	8
17	**2g**	**5**	**5**	**13**	**14**
18	2h	2	0	10	11
19	**2i**	**13**	**10**	**6**	**5**
20	2j	6	7	5	9
21	T	nt	nt	17	19
22	E	18	16	nt	nt

[a]Sample concentration 50 μg/mL.
[b]Zone of growth inhibition measured in millimeter (mm).
T: Tetracycline (50 μg/mL).
E: Erythromycin (50 μg/mL).
0 Value shows no inhibition.
nt**:** Not tested.
Bold value shows highly active compounds from synthesized compounds.

Biology

Anticancer screening of selected synthesized compounds

In vitro anticancer activity was carried out under DTP at National Cancer Institute, Bethesda, USA.[29–31] Among all the compounds, 1a, 1b, 1c, 1d, 1i, 2h, and 2j were selected and initially screened at a single high dose of 10^{-5} M concentration. The entire 60 human cancer cell lines were organized into nine subpanels derived from nine different human cancer types; Leukemia, Non-Small Cell Lung Cancer, Colon, CNS, Melanoma, Ovarian, Renal, Prostate, and Breast cancer cell lines (see Supplementary Material). Output from the single-dose screen is reported as a graph of mean growth percent of the treated cells (Table 2).

All synthesized compounds (1a–j, 2a–j) were submitted to NCI USA, among that 1a-d, 1i, 2h, and 2j were selected for single dose (10^{-5} M) study. The percentage growth inhibition (GI %) of the treated cells at 10^{-5} M concentration of compounds 1a, 1b, 1c, 1d, 1i, 2h, and 2j are presented in Table 1. Tested compounds show moderate to low activity, due to this we have taken average value (<80% GP). Compound 1c have good activity against SNB-75 (CNS), while 2 h has moderate activity against different cell lines NCI-H226 (nscL), SNB-75 (CNS), HS 578T (BC). Overall synthesized compounds are active toward CNS cancer (SNB-75/CNS), renal cancer (UO-31/RC) and its % growth inhibition values are 43.45 and 26.67, respectively.

Antimicrobial activity by minimum inhibitory concentration determination

Synthesized compounds 1a–j, 2a–j tested Minimum inhibitory concentration determination by the agar well diffusion method employed in this work,[32–34] all synthesized compounds activity checked against Gram-positive and Gram-negative bacteria alike *Bacillus megaterium, Bacillus subtilisis, E. coli*, and *Klebsiella* yielded larger zone of inhibition in primary screening then secondary screening done with 10 µg/mL, 25 µg/mL, 50 µg/mL concentrations. Results against **50 µg/mL** concentration shown in Table 3. Checked their zone of growth inhibition in millimeter (mm). Amongst them compound **1c, 1g, 1i, 2g, 2i** was extremely active against Gram-positive bacteria *Bacillus megaterium, Bacillus subtilisis* and growth inhibition value nearer to standard drug erythromycin. Compounds **2a** and **2e** also shows moderate activity compare to erythromycin. Gram-negative bacterial strains in secondary screening compounds **1b, 1i**, and **2g** shows extreme potency compare to standard drug tetracycline and some compounds 1d, 1h, 2h shows moderate activity.

Experimental

Experimental section

All chemicals were purchased from Merck and commercial supplier, all purchased chemicals were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel-G plates (G60 F254 (Merck)) of 0.5 mm thickness, visualizing with ultraviolet light (254 and 365 nm), or with iodine vapor or aq. $KMnO_4$. Melting points were determined using a Buchi B-540 capillary apparatus. IR data were recorded on a Shimadzu FT-IR-8400 instrument using DRS (diffusive reflectance system) method and are reported in cm^{-1} (KBr). NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer (400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR), respectively, in deuterated solvents like DMSO-d6. The control of reaction temperature was monitored by ruby thermometer. Mass spectra were recorded on a Shimadzu GC-MS-QP-2010 mass spectrometer in ESI (70 eV) model using direct inlet probe technique and *m*/*z* is reported in atomic units per elementary charge. Microwave experiments were carried out in an Anton-Paar Monowave 300 Microwave synthesizer using borosilicate glass G10 vial sealed with

PTFE-coated silicone septum. The control of all the reaction temperature was monitored by ruby thermometer.

Procedure for synthesis of isatoic anhydride (Int-1)

To a stirred solution of m-chloroperoxybenzoic acid (68.02 mmol) in THF (20 mL) was added in solution of isatin (34.3 mmol) in THF and 3 h of stirring under ice condition. After that mixture was treated with 10% sodium hydrogen sulfite solution. Product was precipitated in water and extracted with ethyl acetate. Light yellow powder (5.26 g), 95% yield obtained. M.P. °C (242–244).

Procedure for synthesis of 1,2,3,11a-tetrahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (Int-2)

To a stirred solution of **Int-1** (5 g, 30.6 mmol) and L-Proline (3.52 g, 30.6 mmol) in DMSO (20 mL) reaction mixture was heated up to 120 °Cfor 3 h product was precipitated in cold water. Obtained product as off-white color powder (5.89 g), 89% yield obtained. M.P. °C (224–226).

Procedure for synthesis of 10-(prop-2-yn-1-yl)-1,2,3,11a-tetrahydro-5H-benzo[e]pyrrolo[1,2-a][1,4] diazepine-5,11(10H)-dione (Int-3)

To a stirred solution of **Int-2** (3 g, 13.8 mmol) in acetone for 5–10 min at 0 °C, then, addition of propargyl bromide (2.3 mL, 16.6 mmol). Stirred the reaction mass at room temperature for 4 h product is formed in cold water then dry it (3.17 g), 90% off white solid yield obtained. M.P. °C (210–212).

General procedure for synthesis of 10-((1-(substitutedphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-diones (1a–j)

In microwave seal tube **Int-3** (0.1 g, 0.39 mmol) and substituted phenyl azide (0.39 mmol) in water (4 mL), dimethyl formamide (4 mL) and *tert*-butanol (4 mL) followed by sodium ascorbate (15 mg, 0.07 mmol) and dropwise add copper sulfate solution (0.1 mL). The resulting reaction mixture was placed in microwave for 30 to 35 min at 60 °C after completion of reaction product was precipitated in ice water then dry it (0.051 g) average yield were in range of 75–95% and range of synthesized compounds melting point in between 160 and 190 °C.

General procedure for synthesis of N-(substitutedphenyl)-2-(4-((5,11-dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamides (2a-j)

In microwave seal tube, take a stirred solution of **Int-3** (0.1 g, 0.39 mmol) and 2-azido-N-(substituted phenyl)acetamide (0.39 mmol) in water (4 mL), dimethyl formamide (4 mL) and *tert*-butanol (4 mL) followed by addition of sodium ascorbate (15 mg, 0.07 mmol) and dropwise addition of copper sulfate solution (0.1 mL). The resulting reaction mixture was placed in microwave for 30 to 35 min at 60 °C after completion of reaction product was precipitated in ice water then dry it (0.07 g) average yield were in range of 73–91% and range of synthesized compounds melting point in between 188 and 214 °C.

Analytical data for synthesized compounds

Intermediate-1: 2H-benzo[d][1,3]oxazine-2,4(1H)-dione

Yield-95%, Yellow solid, M.P. °C 242–244, ^{1}H NMR (400 MHz, DMSO-d_6) in δ ppm 13.37 (s, 1H), 7.90 (d, $J = 1.3$ Hz, 2H), 7.70 (d, $J = 7.7$ Hz, 1H), 7.55 (d, $J = 8.0$ Hz, 1H).

Intermediate-2: 1,2,3, 11a-tetrahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione

Yield-89%, Off white solid, M.P. °C 224–226, ^{1}H NMR (400 MHz, ^{1}H-NMR (CDCl3) in δ ppm 8.92 (1H, br s, NH), 7.99 (1H, dd, $J = 8.0$, 1.6 Hz), 7.45–7.47 (1H, m), 7.23–7.27 (1H, m), 7.04 (1H, d, $J = 8.0$ Hz), 4.07(1H, d, $J = 6.2$ Hz),3.77–3.82 (1H, m), 3.56–3.63 (1H, m), 2.72–2.80 (1H, m),1.97–2.08 (3H, m).

Intermediate-3: 10-(prop-2-yn-1-yl)-1,2,3,11a-tetrahydro-5H-benzo[e]pyrrolo[1,2-a][1,4] diazepine-5,11(10H)-dione

Yield-90%, Off white solid, M.P. °C 210–212, ^{1}H NMR (400 MHz, ^{1}H-NMR (CDCl3) 7.99 (1H, dd, $J = 8.0$, 1.6 Hz), 7.45–7.47 (1H, m), 7.23–7.27 (1H, m), 7.04 (1H, d, $J = 8.0$ Hz), 4.12 (2H, s) 4.07 (1H, d, $J = 6.2$ Hz),3.77–3.82 (1H, m), 3.56–3.63 (1H, m), 3.08 (1H, s) 2.72–2.80 (1H, m),1.97–2.08 (3H, m).

10-((1-(4-Fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e] pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (1a)

Yield-94%, Off white solid, 190, **IR** (KBr, ν_{max}, cm^{-1}) 3268 (C-H str. in aromatic), 2965.55 (C-H str.), 2880.68 (C-H str.), 1645 (C = O str. in amide), 1597 (Ar. ring skeleton), 1450 (C = C str. in aromatic), 1411 (C-H bend), 1333 (C-N str. in aromatic), 1275 (C-N med.), 1209 (C-N str. in aromatic), 1084 (C-H bend. in aromatic), 1005 (C-F str.), 844 (p-disub. aromatic), **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.42 (d, $J = 1.4$ Hz, 1H), 7.84–7.78 (m, 2H), 7.74 (d, $J = 6.8$ Hz, 1H), 7.66–7.52 (m, 3H), 7.43 (t, $J = 7.3$ Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 5.24 (d, $J = 15.7$ Hz, 1H), 5.06 (d, $J = 15.7$ Hz, 1H), 4.20 (d, $J = 5.8$ Hz, 1H), 3.62 (dd, $J = 11.3$, 5.4 Hz, 1H), 3.43 (dd, $J = 13.7$, 5.7 Hz, 1H), 2.46 (d, $J = 5.4$ Hz, 1H), 2.03–1.86 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.16, 164.03, 154.84, 152.35, 143.86, 139.41, 132.11, 131.25, 131.17, 129.83, 129.61, 125.70, 125.56, 125.50, 125.40, 125.35, 124.65, 124.54, 122.82, 117.23, 117.04, 56.57, 46.27, 43.85, 26.24, 23.28. Anal. Calcd for $C_{21}H_{18}FN_5O_2$: C, 64.44; H, 5.98; N, 17.19. Found: C, 64.41; H, 5.92; N, 17.12. **Mass** (m/z): Cal. mass: 391.41, Found mass: 391.14

10-((1-(2,4-Difluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e] pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (1b)

Off white solid, **IR** (KBr, ν_{max}, cm^{-1}) 3283(C-H str. in aromatic), 2928 (C-H str.), 2872 (C-H str.), 1653 (C = O str. in amide), 1645 (Amide ketone) 1593 (Ar. ring skeleton), 1499 (C = C str. in aromatic), 1410 (C-H bend), 1371 (C-N str. in aromatic), 1273 (C-N med.), 1209 (C-N str. in aromatic), 1086(C-H bend. in aromatic), 1003 (C-F str.),840 (p-disub. aromatic ring), 756 (o-disub. aromatic ring), **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.41 (d, $J = 1.8$ Hz, 1H), 7.87 (td, $J = 8.8$, 5.9 Hz, 1H), 7.79 (d, $J = 8.1$ Hz, 1H), 7.74 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.70–7.60 (m, 2H), 7.34 (t, $J = 7.8$ Hz, 2H), 5.24 (d, $J = 15.8$ Hz, 1H), 5.06 (d, $J = 15.7$ Hz, 1H), 4.26–4.14 (m, 1H), 3.71–3.53 (m, 1H), 3.43 (dd, $J = 13.7$, 5.8 Hz, 1H), 2.46 (d, $J = 5.8$ Hz, 1H), 2.05–1.85 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.14, 164.03, 163.38, 163.26, 162.26, 160.90, 160.78, 155.54, 155.41, 153.02, 152.89, 143.83, 139.41, 132.10, 129.82, 129.61, 127.45, 127.35, 125.55, 125.49, 122.80, 121.64, 121.60, 121.52, 121.49, 112.81, 112.77, 112.58, 112.54, 105.92, 105.68, 105.65, 105.41, 56.56, 46.26, 43.86, 35.73, 30.71, 26.23, 23.27. **Anal. Calcd** for $C_{21}H_{17}F_2N_5O_2$: C,

61.61; H, 4.19; N, 17.11. **Found**: C, 61.58; H, 4.17; N, 17.04, **Mass** (*m*/*z*): Cal. mass: 409.40, Found mass: 409.14.

10-((1-(2-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e] pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (1c)

Off white solid, **IR** (KBr, ν_{max}, cm^{-1}) 3278 (C-H Str. in aromatic), 2967 (C-H str.), 2876 (C-H str.), 1647 (C = O str. in amide), 1597 (Ar. ring skeleton), 1499 (C = C str. in aromatic), 1402 (C-H bend), 1331 (C-N str. in aromatic), 1261 (C-N med.), 1207 (C-N str. in aromatic), 1082 (C-H bend. in aromatic), 742 (o-disub. aromatic), 688 (C-Cl str.), **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.34 (s, 1H), 7.94 (s, 1H), 7.75 (dd, J = 7.3, 5.1 Hz, 2H), 7.73 (dd, J = 3.5, 1.4 Hz, 1H), 7.66–7.53 (m, 4H), 7.33 (t, J = 7.2 Hz, 1H), 5.17 (dd, J = 15.7 Hz, 2H), 4.24–4.15 (m, 1H), 3.60 (dt, J = 10.6, 5.1 Hz, 1H), 2.49–2.43 (m, 1H), 2.01–1.86 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.12, 164.03, 162.32, 143.22, 139.23, 134.32, 132.09, 131.60, 130.50, 129.94, 129.58, 128.42, 128.36, 128.18, 126.14, 125.52, 122.92, 56.59, 46.27, 43.63, 35.76, 30.73, 26.23, 23.27. **Anal. Calcd** for $C_{21}H_{18}ClN_5O_2$: C, 61.84; H, 4.45; N, 17.17. **Found**: C, 61.80; H, 4.39; N, 17.13, **Mass** (*m*/*z*): Cal. mass: 407.86, Found mass: 407.11.

10-((1-(4-Bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e] pyrrolo [1,2-a][1,4]diazepine-5,11(10H)-dione (1d)

White solid, **IR** (KBr, ν_{max}, cm^{-1}) 3261 (C-H str. in aromatic), 2862 (C-H str.), 1639 (C = O str. in amide), 1594 (Ar. ring skeleton), 1468 (C = C str. in aromatic), 1507 (C-H bend), 1321 (C-N str. in aromatic), 1266 (C-N med.), 1208 (C-N str. in aromatic), 1095 (C-H bend. in aromatic), 838(p-disub. aromatic ring), 693 (C-Br str.), **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.60 (s, 1H), 7.80 (d, J = 8.9 Hz, 2H), 7.78–7.70 (m, 4H), 7.60–7.50 (m, 1H), 7.32 (t, J = 7.4 Hz, 1H), 5.35–5.12 (m, 1H), 5.00 (d, J = 15.8 Hz, 1H), 4.24–4.11 (m, 1H), 3.58 (dt, J = 10.5, 5.1 Hz, 1H), 3.42 (d, J = 8.3 Hz, 1H), 2.41 (s, 1H), 2.03–1.89 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.09, 164.07, 144.65, 139.46, 135.64, 132.75, 132.14, 129.73, 129.60, 125.49, 122.72, 122.17, 121.85, 121.29, 56.58, 46.30, 44.06, 26.24, 23.28. **Anal. Calcd** for $C_{21}H_{18}BrN_5O_2$: C, 55.76; H, 4.01; N, 15.48. **Found**: C, 55.70; H, 3.97; N, 15.44, **Mass** (*m*/*z*): Cal. mass: 452.31, Found mass: 451.06 and 453.06.

10-((1-Phenyl-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e]pyrrolo [1,2-a] [1,4]diazepine-5,11(10H)-dione (1e)

White solid, **IR** (KBr, ν_{max}, cm^{-1}) 3281 (C-H str. in aromatic), 2876 (C-H str.), 1644 (C = O str. in amide), 1592 (Ar. ring skeleton), 1491(C = C str. in aromatic), 1275 (C-N str. in aromatic), 1261(C-N med.), 1202 (C-N str. in aromatic), 1082 (C-H bend. in aromatic). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.71 (s, 1H), 7.85 (d, J = 8.9 Hz, 2H), 7.81–7.72 (m, 5H), 7.64–7.58 (m, 1H), 7.33 (t, J = 7.4 Hz, 1H), 5.36–5.14 (m, 1H), 5.00 (d, J = 15.8 Hz, 1H), 4.26–4.12 (m, 1H), 3.62 (dt, J = 10.7, 5.2 Hz, 1H), 3.45 (d, J = 8.5 Hz, 1H), 2.46 (s, 1H), 2.05–1.85 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.24, 164.56, 162.42, 143.86, 139.24, 134.32, 132.09, 131.69, 130.50, 129.54, 128.36, 126.13, 125.76, 122.00, 56.59, 46.27, 43.63, 35.76, 30.73, 26.24, 23.32. **Anal. Calcd** for $C_{21}H_{19}N_5O_2$: C, 67.55; H, 5.13; N, 18.76, **Found**: C, 67.51; H, 5.09; N, 18.70. **Mass** (*m*/*z*): Cal. mass: 373.42, Found mass: 373.15.

10-((1-(p-Tolyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e]pyrrolo [1,2-a][1,4]diazepine-5,11(10H)-dione (1f)

Off white solid, **IR** (KBr, ν_{max}, cm^{-1}) 3261 (C-H Str. in aromatic), 2968 (C-H str. in alkane), 2887 (C-H Str.), 1647 (C = O str. in amide), 1595 (Ar. ring skeleton), 1454 (C = C str. in aromatic), 1408 (C-H bend), 1333 (C-N str. in aromatic), 1276 (C-N med.), 1209 (C-N str. in

aromatic), 1109 (C-H bend. in aromatic), 831 (p-disub. aromatic ring). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.54 (s, 1H), 7.82 (d, $J = 8.7$ Hz, 2H), 7.72–7.64 (m, 4H), 7.58–7.52 (m, 1H), 7.31 (t, $J = 7.3$ Hz, 1H), 5.25–5.10 (m, 1H), 5.00 (d, $J = 15.8$ Hz, 1H), 4.28–4.10 (m, 1H), 3.64 (dt, $J = 10.8$, 5.3 Hz, 1H), 3.41 (d, $J = 8.4$ Hz, 1H), 3.12 (s, 3H) 2.42 (s, 1H), 2.02–1.81 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 168.27, 165.42, 162.18, 143.74, 138.27, 133.31, 132.10, 131.55, 130.50, 129.44, 127.30, 126.41, 125.54, 122.25, 56.78, 46.74, 43.13, 35.14, 30.36, 26.24, 23.32, 21.89. **Anal. Calcd** for $C_{22}H_{21}N_5O_2$: C, 68.20; H, 5.48; N, 18.08. **Found**: C, 68.16; H, 5.42; N, 18.01, **Mass** (m/z): Cal. mass: 387.44, Found mass: 387.17.

10-((1-(3-Bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e] pyrrolo [1,2-a][1,4]diazepine-5,11(10H)-dione (1g)

Light yellow solid, **IR** (KBr, ν_{max}, cm^{-1}) 3269 (C-H str. in aromatic), 2866 (C-H str.), 1645 (C = O str. in amide), 1597 (Ar. ring skeleton), 1460 (C = C str. in aromatic), 1501 (C-H bend), 1327 (C-N str. in aromatic), 1261 (C-N med.), 1199 (C-N str. in aromatic), 1088 (C-H bend. in aromatic), 760(m-disub. aromatic ring), 687 (C-Br str.). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.34 (s, 1H), 7.94 (s, 1H), 7.75 (dd, $J = 7.3$, 5.1 Hz, 2H), 7.73 (dd, $J = 3.5$, 1.4 Hz, 1H), 7.66–7.53 (m, 4H), 7.33 (t, $J = 7.2$ Hz, 1H), 5.17 (dd, $J = 15.7$ Hz, 2H), 4.24–4.15 (m, 1H), 3.60 (dt, $J = 10.6$, 5.1 Hz, 1H), 2.49–2.43 (m, 1H), 2.01–1.86 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.25, 164.44, 162.26, 143.49, 139.18, 134.76, 132.01, 131.84, 130.54, 129.74, 128.43, 126.23, 125.64, 122.92, 56.59, 46.27, 43.63, 35.76, 30.73, 26.23, 23.27. **Anal. Calcd** for $C_{21}H_{18}BrN_5O_2$: C, 55.76; H, 4.01; N, 15.48. **Found**: C, 55.72.16; H, 3.95; N, 15.43, **Mass** (m/z): Cal. mass: 452.29, Found mass: 451.04 and 453.04.

10-((1-(4-Ethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e] pyrrolo [1,2-a][1,4]diazepine-5,11(10H)-dione (1h)

Off white solid, **IR** (KBr, ν_{max}, cm^{-1}) 3268 (C-H Str. in aromatic), 2969 (C-H str. in alkane), 2888 (C-H Str.), 1649 (C = O str. in amide), 1595 (Ar. ring skeleton), 1454 (C = C str. in aromatic), 1408 (C-H bend), 1334 (C-N str. in aromatic), 1277 (C-N med.), 1209 (C-N str. in aromatic), 1107 (C-H bend. in aromatic), 831 (p-disub. aromatic ring). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.54 (s, 1H), 7.82 (d, $J = 8.7$ Hz, 2H), 7.72–7.64 (m, 4H), 7.58–7.52 (m, 1H), 7.31 (t, $J = 7.3$ Hz, 1H), 5.25–5.10 (m, 1H), 5.00 (d, $J = 15.8$ Hz, 1H), 4.28–4.10 (m, 1H), 3.64 (dt, $J = 10.8$, 5.3 Hz, 1H), 3.41 (d, $J = 8.4$ Hz, 1H), 3.12 (q, 2H), 2.42 (s, 1H), 2.02–1.81 (m, 3H) 1.38 (t, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 168.27, 165.42, 162.18, 143.74, 138.27, 133.31, 132.10, 131.55, 130.50, 129.44, 127.30, 126.41, 125.54, 122.25, 56.78, 46.74, 43.13, 35.14, 30.36, 27.65, 26.24, 23.32, 21.89. **Anal. Calcd** for $C_{21}H_{18}BrN_5O_2$: C, 55.76; H, 4.01; N, 15.48. **Found**: C, 55.72.16; H, 3.95; N, 15.43, **Mass** (m/z): Cal. mass: 401.47, Found mass: 401.20.

10-((1-(3-Chloro-2-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (1i)

Off white solid, **IR** (KBr, ν_{max}, cm^{-1}) 3275 (C-H str. in aromatic), 2965 (C-H str.), 2857 (C-H str.), 1645 (C = O str. in amide), 1594 (Ar. ring skeleton), 1491 (C = C str. in aromatic), 1405 (C-H bend), 1329 (C-N str. in aromatic), 1269 (C-N med.), 1205 (C-N str. in aromatic), 1082 (C-H bend. in aromatic), 795 (o-disub. aromatic ring), 761 (m- disub. aromatic ring), 684.75 (C-Cl str.). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.15 (s, 1H), 7.74 (s, 1H), 7.60 (dd, $J = 7.3$, 5.1 Hz, 2H), 7.55 (dd, $J = 3.3$, 1.2 Hz, 1H), 7.50–7.40 (m, 4H), 5.09 (dd, $J = 13.7$ Hz, 2H), 4.20–4.10 (m, 1H), 3.60 (dt, $J = 10.6$, 5.1 Hz, 1H), 2.94 (s, 3H), 2.49–2.43 (m, 1H), 2.01–1.86 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 168.01, 164.45, 162.78, 143.98, 139.16, 134.68, 132.39, 131.76, 130.78, 129.24, 128.63, 126.10, 125.77, 122.99, 56.45, 46.56, 43.47, 35.83, 30.23,

30.24, 26.23, 23.27. **Anal. Calcd** for $C_{22}H_{20}ClN_5O_2$: C, 62.63; H, 4.78; N, 16.60. **Found**: C, 62.59; H, 4.13; N, 16.54, **Mass** (*m*/*z*): Cal. mass: 421.89, Found mass: 421.13 and 423.13.

10-((1-(4-Nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-1,2,3,11a-tetrahydro-5H-benzo[e] pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (1j)

Light yellow solid, **IR** (KBr, ν_{max}, cm^{-1}) 3275 (C-H str. in aromatic), 2860 (C-H str.), 1652 (C = O str. in amide), 1603 (Ar. ring skeleton), 1497 (C = C str. in aromatic), 1417 (N = O str.), 1327 (C-N str. in aromatic), 1269 (C-N med.), 1204 (C-N str. in aromatic), 1070 (C-H bend. in aromatic), 772 (o-disub. aromatic ring). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.40 (s, 1H), 8.01 (s, 1H), 7.80 (dd, J = 7.4, 5.3 Hz, 2H), 7.78 (dd, J = 3.4, 1.4 Hz, 1H), 7.66–7.53 (m, 4H), 7.33 (t, J = 7.2 Hz, 1H), 5.17 (dd, J = 15.7 Hz, 2H), 4.24–4.15 (m, 1H), 3.60 (dt, J = 10.6, 5.1 Hz, 1H), 2.49–2.43 (m, 1H), 2.01–1.86 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.49, 164.89, 162.46, 143.29, 139.94, 134.74, 132.36, 131.90, 130.70, 129.73, 128.24, 126.19, 125.95, 122.79, 56.65, 46.47, 43.61, 35.96, 30.89, 26.75, 23.46. **Anal. Calcd** for $C_{21}H_{18}N_6O_4$: C, 60.28; H, 4.34; N, 20.09. **Found**: C, 60.21; H, 4.28; N, 20.03, **Mass** (*m*/*z*): Cal. mass: 418.41, Found mass: 418.14.

2-(4-((5,11-Dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-N-(4-fluorophenyl)acetamide (2a)

White solid, **IR** (KBr, ν_{max}, cm^{-1}) 3392 (N-H str.), 3183 (C-H str. in aromatic), 2962 (C-H str.), 2874 (C-H str.), 1647 (C = O str. in amide), 1593 (N-H bend.) 1535 (Ar. ring skeleton), 1498 (C = C str. in aromatic), 1415 (C-H bend), 1369 (C-N str. in aromatic), 1290 (C-N med.), 1220 (C-N str. in aromatic), 1082(C-H bend. in aromatic), 1037 (C-F str.), 815 (p- disub. aromatic ring), **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.67 (s, 1H) 8.34 (d, J = 1.4 Hz, 1H), 7.79–7.76 (m, 2H), 7.70 (d, J = 6.7 Hz, 1H), 7.63–7.50 (m, 3H), 7.39 (t, J = 7.2 Hz, 1H), 7.30 (t, J = 7.3 Hz, 1H), 5.22 (d, J = 15.4 Hz, 1H), 5.04 (d, J = 15.5 Hz, 1H), 4.47 (s, 2H) 4.15 (d, J = 5.8 Hz, 1H), 3.59 (dd, J = 11.0, 5.1 Hz, 1H), 3.40 (dd, J = 13.5, 5.5 Hz, 1H), 2.44 (d, J = 5.2 Hz, 1H), 2.01–1.89 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.78, 164.03, 162.20. 159.34, 154.84, 152.35, 143.86, 139.41, 132.11, 131.25, 129.61, 127.54, 125.70, 124.65, 122.82, 117.23, 107.65, 56.57, 46.27, 43.85, 34.56, 26.24, 23.28. **Anal. Calcd** for $C_{23}H_{21}ClN_6O_3$: C, 59.42; H, 4.55; N, 18.08. **Found**: C, 59.38; H, 4.50; N, 18.03, **Mass** (*m*/*z*): Cal. mass: 448.46, Found mass: 448.17.

N-(2,4-difluorophenyl)-2-(4-((5,11-dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a] [1,4]diazepin-10(5H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (2b)

White solid, **IR** (KBr, ν_{max}, cm^{-1}) 3278 (C-H str. in aromatic), 2918 (C-H str.), 2863 (C-H str.), 1649 (C = O str. in amide), 1590 (Ar. ring skeleton), 1488 (C = C str. in aromatic), 1408 (C-H bend), 1371 (C-N str. in aromatic), 1272 (C-N med.), 1205 (C-N str. in aromatic), 1080 (C-H bend. in aromatic), 1003.02 (C-F str.), 810 (p- disub. aromatic ring) 756.12 (o- disub. aromatic ring).) , **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.77 (s, 1H), 8.42 (s, 1H), 7.85 (td, J = 8.4, 5.7 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.73 (dd, J = 7.6, 1.5 Hz, 1H), 7.70–7.60 (m, 2H), 7.34 (t, J = 7.8 Hz, 2H), 5.24 (d, J = 15.6 Hz, 1H), 5.06 (d, J = 15.7 Hz, 1H), 4.52 (s, 2H) 4.26–4.14 (m, 1H), 3.71–3.53 (m, 1H), 3.41 (dd, J = 13.4, 5.6 Hz, 1H), 2.40 (d, J = 5.5 Hz, 1H), 2.05–1.85 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.14, 163.38, 162.26, 160.90, 155.41, 153.02, 152.89, 143.83, 139.41, 132.10, 129.61, 127.35, 125.49, 122.80, 121.75, 112.81, 105.92, 56.56, 46.26, 43.86, 35.73, 26.23, 23.27. **Anal. Calcd** for $C_{23}H_{20}F_2N_6O_3$: C, 59.22; H, 4.32; N, 18.02. **Found**: C, 59.18; H, 4.25; N, 17.97, **Mass** (*m*/*z*): Cal. mass: 466.55, Found mass: 466.24.

N-(2-chlorophenyl)-2-(4-((5,11-dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a] [1,4] diazepin-10(5H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (2c)

White solid, **IR** (KBr, ν_{max}, cm^{-1}) 3273 (C-H str. in aromatic), 2968 (C-H str.), 2856 (C-H str.), 1645 (C = O str. in amide), 1595 (Ar. ring skeleton), 1491 (C = C str. in aromatic), 1404 (C-H bend), 1329 (C-N str. in aromatic), 1269 (C-N med.), 1205 (C-N str. in aromatic), 1080 (C-H bend. in aromatic), 796(o-disub. aromatic ring), 745 (o- disub. aromatic ring), 687 (C-Cl str.). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.92 (s, 1H), 8.41 (d, J = 1.8 Hz, 1H), 7.87 (td, J = 8.8, 5.9 Hz, 2H), 7.79 (d, J = 8.1 Hz, 1H), 7.74 (dd, J = 7.8, 1.5 Hz, 1H), 7.70–7.60 (m, 2H), 7.34 (t, J = 7.8 Hz, 2H), 5.24 (d, J = 15.8 Hz, 1H), 5.06 (d, J = 15.7 Hz, 1H), 4.52 (s, 2H) 4.26–4.14 (m, 1H), 3.71–3.53 (m, 1H), 3.43 (dd, J = 13.7, 5.8 Hz, 1H), 2.46 (d, J = 5.8 Hz, 1H), 2.05–1.85 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.94, 164.03, 163.38, 162.26, 160.78, 155.54, 153.02, 143.83, 139.52, 132.10, 129.61, 127.35, 125.73, 122.87, 121.60, 112.49, 105.35, 56.50, 46.21, 43.75, 35.83, 26.47, 23.19. **Anal. Calcd** for $C_{23}H_{21}ClN_6O_3$: C, 59.42; H, 4.55; N, 18.08. **Found**: C, 59.38; H, 4.49; N, 18.02, **Mass** (m/z): Cal. mass: 464.91, Found mass: 464.14 and 466.13.

N-(4-bromophenyl)-2-(4-((5,11-dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a] [1,4] diazepin-10(5H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (2d)

Off White Solid, **IR** (KBr, ν_{max}, cm^{-1}) 32658 (C-H str. in aromatic), 2855 (C-H str.), 1645 (C = O str. in amide), 15980 (Ar. ring skeleton), 1465 (C = C str. in aromatic), 1499 (C-H bend), 1325 (C-N str. in aromatic), 1267 (C-N med.), 1207 (C-N str. in aromatic), 1094 (C-H bend. in aromatic), 834(p-disub. aromatic ring), 668 (C-Br str.). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.69 (s, 1H) 8.55 (d, J = 1.4 Hz, 1H), 7.12–7.16 (m, 2H), 7.72 (d, J = 6.6 Hz, 1H), 7.62–7.55 (m, 3H), 7.41 (t, J = 7.2 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 5.22 (d, J = 15.5 Hz, 1H), 5.05 (d, J = 15.1 Hz, 1H), 4.51 (s, 2H) 4.18 (d, J = 5.6 Hz, 1H), 3.64 (dd, J = 11.4, 5.4 Hz, 1H), 3.43 (dd, J = 13.7, 5.7 Hz, 1H), 2.42 (d, J = 5.3 Hz, 1H), 2.09–1.90 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.75, 164.19, 157.74, 152.65, 143.25, 139.29, 135.74, 132.11, 131.25, 129.83, 127.70, 126.49, 125.35, 124.65, 122.82, 121.78, 117.04, 56.57, 46.27, 43.85, 35.69, 26.24, 23.28. **Anal. Calcd** for $C_{23}H_{21}BrN_6O_3$: C, 54.23; H, 4.16; N, 16.50. **Found**: C, 54.19; H, 4.11; N, 16.46, **Mass** (m/z): Cal. mass: 509.36, Found mass: 508.09.14 and 510.10.

2-(4-((5,11-Dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-N-phenylacetamide (2e)

White solid **IR** (KBr, ν_{max}, cm^{-1}) 3245 (C-H str. in aromatic), 2871 (C-H str.), 1644 (C = O str. in amide), 1588 (Ar. ring skeleton), 1472 (C = C str. in aromatic), 1275 (C-N str. in aromatic), 1264(C-N med.), 1189 (C-N str. in aromatic), 1081 (C-H bend. in aromatic). .). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.71 (s, 1H), 8.13 (d, J = 1.8 Hz, 1H), 7.82 (td, J = 8.6, 5.7 Hz, 2H), 7.75 (d, J = 7.9 Hz, 1H), 7.70 (dd, J = 7.6, 1.5 Hz, 1H), 7.68–7.27 (m, 5H), 5.20 (d, J = 15.4 Hz, 1H), 5.06 (d, J = 15.7 Hz, 1H), 4.52 (s, 2H) 4.22–4.10 (m, 1H), 3.69–3.53 (m, 1H), 3.44 (dd, J = 13.5, 5.6 Hz, 1H), 2.75 (d, J = 5.2 Hz, 1H), 2.13–1.90 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.14, 164.03, 162.26, 160.90, 155.41, 153.02, 152.89, 143.83, 139.41, 132.10, 129.82, 127.35, 125.49, 122.80, 121.60, 112.54, 105.92, 56.56, 46.26, 43.86, 35.73, 26.23, 23.27. **Anal. Calcd** for $C_{23}H_{22}N_6O_3$: C, 64.18; H, 5.15; N, 19.52. **Found**: C, 64.14; H, 5.11; N, 19.48, **Mass** (m/z): Cal. mass: 430.47, Found mass: 430.18.

2-(4-((5,11-Dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-N-(p-tolyl)acetamide (2f)

White solid, **IR** (KBr, ν_{max}, cm^{-1}) 3258 (C-H Str. in aromatic), 2955 (C-H str. in alkane), 2874 (C-H Str.), 1645 (C = O str. in amide), 1501 (Ar. ring skeleton), 1442 (C = C str. in aromatic),

1432 (C-H bend), 1325 (C-N str. in aromatic), 1245 (C-N med.), 1198 (C-N str. in aromatic), 1101 (C-H bend. in aromatic), 823 (p-disub. aromatic ring). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.89 (s, 1H) 8.36 (d, $J = 1.2$ Hz, 1H), 7.84–7.78 (m, 2H), 7.72 (d, $J = 6.8$ Hz, 1H), 7.68–7.56 (m, 3H), 7.43 (t, $J = 7.3$ Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 5.24 (d, $J = 15.7$ Hz, 1H), 5.06 (d, $J = 15.7$ Hz, 1H), 4.51 (s, 2H) 4.20 (d, $J = 5.8$ Hz, 1H), 3.62 (dd, $J = 11.3$, 5.4 Hz, 1H), 3.43 (dd, $J = 13.7$, 5.7 Hz, 1H), 2.46 (d, $J = 5.4$ Hz, 1H), 2.30 (s, 3H) 2.03–1.86 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.51, 164.72, 160.36, 153.84, 152.35, 143.86, 141.32, 139.41, 134.11, 131.46, 129.61, 127.70, 126.56, 125.50, 124.65, 124.54, 122.82, 117.04, 56.57, 46.27, 43.85, 37.21, 26.63, 24.47. **Anal. Calcd** for $C_{24}H_{24}N_6O_3$: C, 68.85; H, 5.44; N, 18.91. **Found**: C, 68.80; H, 5.39; N, 18.87, **Mass** (m/z): Cal. mass: 444.50, Found mass: 444.19.

N-(3-bromophenyl)-2-(4-((5,11-dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a] [1,4] diazepin-10(5H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (2g)

Off White Solid, IR (KBr, ν_{max}, cm^{-1}) 3269.45 (C-H str. in aromatic), 2866.32 (C-H str.), 1645.33 (C = O str. in amide), 1597.11 (Ar. ring skeleton), 1460.46 (C = C str. in aromatic), 1500.67 (C-H bend), 1327.05 (C-N str. in aromatic), 1261.49 (C-N med.), 1199.76 (C-N str. in aromatic), 1087.89 (C-H bend. in aromatic), 759.98 (m-disub. aromatic ring), 686.68 (C-Br str.).) . **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.45 (s, 1H), 8.12 (d, $J = 1.6$ Hz, 1H), 7.68 (td, $J = 8.5$, 5.3 Hz, 2H), 7.74 (d, $J = 7.8$ Hz, 1H), 7.69 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.63–7.57 (m, 2H), 7.28 (t, $J = 7.7$ Hz, 2H), 5.24 (d, $J = 15.8$ Hz, 1H), 5.06 (d, $J = 15.7$ Hz, 1H), 4.52 (s, 2H) 4.23–4.17 (m, 1H), 3.68–3.56 (m, 1H), 3.40 (dd, $J = 13.3$, 5.0 Hz, 1H), 2.41 (d, $J = 5.5$ Hz, 1H), 2.10–1.91 (m, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 168.21, 164.09, 163.43, 160.14, 154.54, 153.02, 152.19, 145.83, 138.41, 131.56, 129.41, 127.73, 124.55, 122.80, 121.41, 110.81, 106.71, 56.94, 45.52, 42.72, 35.39, 26.98, 23.86. **Anal. Calcd** for $C_{23}H_{21}BrN_6O_3$: C, 54.23; H, 4.16; N, 16.50. **Found**: C, 54.20; H, 4.12; N, 16.47, **Mass** (m/z): Cal. mass: 509.34, Found mass: 508.10 and 510.11.

N-(3-chlorophenyl)-2-(4-((5,11-dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a] [1,4] diazepin-10(5H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (2h)

White solid, **IR** (KBr, ν_{max}, cm^{-1}) 3352 (N-H Str.), 3192 (C-H Str. in aromatic), 3130 (C-H str.), 2964 (C-H str.), 1680 (C = O str. in amide), 1654 (N-H bend.) 1577 (Ar. ring skeleton), 1487 (C = C str. in aromatic), 1402.30 (C-H bend), 1325 (C-N str. in aromatic), 1209 (C-N med.), 1180 (C-N str. in aromatic), 1084 (C-H bend. in aromatic), 869 (p-disub. aromatic), 681 (C-Cl str.), **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.83 (s, 1H), 8.27 (d, $J = 1.6$ Hz, 1H), 7.90 (td, $J = 8.5$, 5.7 Hz, 2H), 7.79 (d, $J = 8.1$ Hz, 1H), 7.74 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.70–7.60 (m, 2H), 7.34 (t, $J = 7.8$ Hz, 2H), 5.24 (d, $J = 15.8$ Hz, 1H), 5.06 (d, $J = 15.7$ Hz, 1H), 4.52 (s, 2H) 4.26–4.14 (m, 1H), 3.71–3.53 (m, 1H), 3.43 (dd, $J = 13.7$, 5.8 Hz, 1H), 2.46 (d, $J = 5.8$ Hz, 1H), 2.05–1.85 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 169.14, 164.03, 163.38, 163.26, 162.26, 160.90, 160.78, 155.54, 155.41, 153.02, 152.89, 143.83, 139.41, 132.10, 129.82, 129.61, 127.45, 127.35, 125.55, 125.49, 122.80, 121.64, 121.60, 121.52, 121.49, 112.81, 112.77, 112.58, 112.54, 105.92, 105.68, 105.65, 105.41, 56.56, 46.26, 43.86, 35.73, 26.23, 23.27. **Anal. Calcd** for $C_{23}H_{21}ClN_6O_3$: C, 59.42; H, 4.55; N, 18.08. **Found**: C, 59.39; H, 4.51; N, 18.03. **Mass** (m/z): Cal. mass: 464.91, Found mass: 464.14 and 66.13.

2-(4-((5,11-Dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-10(5H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-N-(4-nitrophenyl)acetamide (2i)

Yellow Solid, **IR** (KBr, ν_{max}, cm^{-1}) 3263 (C-H str. in aromatic), 2869 (C-H str.), 1657 (C = O str. in amide), 1587 (Ar. ring skeleton), 1494 (C = C str. in aromatic), 1410 (N = O str.), 1317 (C-N str. in aromatic), 1269 (C-N med.), 1207 (C-N str. in aromatic), 1068 (C-H bend. in aromatic),

732 (m-disub. aromatic ring). **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.75 (s, 1H), 8.48 (d, $J = 1.8$ Hz, 1H), 7.69 (td, $J = 8.4$, 5.7 Hz, 2H), 7.66 (d, $J = 7.9$ Hz, 1H), 7.74 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.71–7.62 (m, 2H), 7.30 (t, $J = 7.6$ Hz, 2H), 5.21 (d, $J = 15.4$ Hz, 1H), 5.1 (d, $J = 14.7$ Hz, 1H), 4.46 (s, 2H) 4.26–4.14 (m, 1H), 3.69–3.50 (m, 1H), 3.41 (dd, $J = 12.9$, 5.7 Hz, 1H), 2.44 (d, $J = 5.6$ Hz, 1H), 2.05–1.85 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 168.44, 164.15, 163.02, 162.26, 160.78, 155.41, 153.02, 143.83, 139.41, 132.10, 129.61, 127.35, 125.49, 122.80, 121.64, 112.81, 105.41, 56.56, 46.26, 43.86, 35.73, 26.23, 23.27. **Anal. Calcd** for $C_{23}H_{21}ClN_6O_3$: C, 58.10; H, 4.45; N, 20.62. **Found**: C, 58.07; H, 4.42; N, 20.58, **Mass** (m/z): Cal. mass: 475.47, Found mass: 475.16.

N-(5-chloropyridin-2-yl)-2-(4-((5,11-dioxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo [1,2-a] [1,4]diazepin-10(5H)-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (2j)

Off white solid, **IR** (KBr, ν_{max}, cm^{-1}) 3452 (N-H Str.), 3192 (C-H Str. in aromatic), 3130 (C-H str.), 3057 (C-H str.), 1680 (C = O str. in amide), 1650 (N-H bend.) 1589 (Ar. ring skeleton), 1477 (C = C str. in aromatic), 1415 (C-H bend), 1383 (C-N str. in aromatic), 1217 (C-N med.), 1172 (C-N str. in aromatic), 1074 (C-H bend. in aromatic), 883 (p-disub. aromatic), 628 (C-Cl str.), **^{1}H NMR** (400 MHz, DMSO-d_6) in δ ppm 8.77 (s, 1H), 8.42 (s, 1H), 7.85 (td, $J = 8.4$, 5.7 Hz, 1H), 7.77 (d, $J = 8.0$ Hz, 1H), 7.73 (dd, $J = 7.6$, 1.5 Hz, 1H), 7.70–7.60 (m, 2H), 7.34 (t, $J = 7.8$ Hz, 2H), 5.24 (d, $J = 15.6$ Hz, 1H), 5.06 (d, $J = 15.7$ Hz, 1H), 4.52 (s, 2H) 4.26–4.14 (m, 1H), 3.71–3.53 (m, 1H), 3.41 (dd, $J = 13.4$, 5.6 Hz, 1H), 2.40 (d, $J = 5.5$ Hz, 1H), 2.05–1.85 (m, 3H), **^{13}C NMR** (101 MHz, DMSO-d_6) in δ ppm 168.85, 163.74, 162.03, 160.45, 155.47, 153.29, 152.41, 143.67, 139.19, 132.43, 129.38, 127.72, 125.68, 122.74, 121.52, 112.81, 105.41, 56.47, 46.89, 43.76, 35.84, 26.34, 23.98. **Anal. Calcd** for $C_{22}H_{20}ClN_7O_3$: C, 56.72; H, 4.33; N, 21.05. **Found**: C, 56.69; H, 4.30; N, 21.01, **Mass** (m/z): Cal. mass: 465.90, Found mass: 465.14 and 467.13.

Conclusion

In conclusion, we designed and developed pyrrolobenzodiazepine derivatives possessing 1,2,3-triazoles moiety by click chemistry synthesis aspect with help of Cu(I)-catalyzed azide-alkyne cycloaddition reaction under microwave irradiation. Final adducts were fusion of two active pharmacophore with significant yield. Finally, the application of synthesized molecules has been screening for anticancer and antimicrobial activities. Parallel kind of pyrrolobenzodiazepine and click chemistry derivatives are under development in our laboratory.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

1. B. K. Ishwar and K. Abhishek, "Synthesis and anti-Inflammatory Activity of Some Novel 1,5 Benzodiazepine Derivatives," *Asian Journal of Pharmaceutical and Clinical Research* 9, no. 4 (2016): 63–6.
2. N. O. Terence, A. Redouane, E. G. Mostafa, E. O. Latyfa, E. J. Meryem, C. Laila, C. Yahia, A. Katim, and Z. Amina, "Analgesic and Antioxidant Activities of 4-Phenyl-1,5-Benzodiazepin-2-One and Its Long Carbon Chains Derivatives," *Journal of Chemistry* 2019 (2019): 9043570.
3. G. O. Juan and A. K. William, "The Role of Benzodiazepines in the Treatment of Epilepsy," *Current Treatment Options in Neurology* 18, no. 18 (2019): 1–11.
4. J. S. David, "The Pharmacology and Mechanisms of Action of New Generation, Non-Benzodiazepine Hypnotic Agents," *CNS Drugs* 18, no. 1 (2004): 9–15.
5. W. Leimgruber, V. Stefanović, F. Schenker, A. Karr, and J. Berger, "Isolation and Characterization of Anthramycin, a New Antitumor Antibiotic," *Journal of the American Chemical Society* 87, no. 24 (1965): 5791–3.
6. A. Dyeison and E. T. David, "Synthesis of DNA-Interactive Pyrrolo[2,1-c][1,4]Benzodiazepines (PBDs)," *Chemical Reviews* 111 (2011): 2815–64.
7. H. H. Laurence, R. Teri, E. T. David, R. L. David, G. H. Kenneth, P. H. Robert, R. E. John, G. Gregory Jr., and F. F. Leo, "Pyrrolo[1,4]Benrodiazepine Antitumor Antibiotics: Relationship of DNA Alkylation and Sequence Specificity to the Biological Activity of Natural and Synthetic Compounds," *Chemical Research in Toxicology* 1 (1988): 258–68.
8. D. T. Moses and K. Samuel, "Refuin: Non-Cytotoxic Carcinostatic Compound Proliferated by a Thermophilic Actinomycete," *Nature* 199 (1963): 501.
9. K. Ahmed, G. Ramakrishna, V. L. Nayak, P. Raju, A. V. Subba Rao, A. Viswanath, M. V. Vishnuvardhan, R. Sistla, and G. Srinivas, "Design and Synthesis of Benzo[c,d]Indolone-Pyrrolobenzodiazepine Conjugates as Potential Anticancer Agents," *Bioorganic & Medicinal Chemistry* 20, no. 2 (2012): 789–800.
10. A. H. John and H. Daniel, "Small Molecule Drugs – Optimizing DNA Damaging Agent-Based Therapeutics," *Current Opinion in Pharmacology* 12 (2012): 398–402.
11. A. M. Burger, P. M. Loadman, D. E. Thurston, R. Schultz, H. H. Fiebig, and M. C. Bibby, "Preclinical Pharmacology of the Pyrrolobenzodiazepine (PBD) Monomer DRH-417 (NSC 709119)," *Journal of Chemotherapy* 19, no. 1 (2007): 66–78.
12. A. H. John, "The Development of Pyrrolobenzodiazepines as Antitumour Agents," *Expert Opinion on Investigational Drugs* 20 (2011): 733–44.
13. P. H. Robert, M. H. Sidney, L. R. Vincent, J. M. Ian, and H. H. Laurence, "DNA Sequence Specificity of the Pyrrolo [1,4]Benzodiazepine Antitumor Antibiotics. Methidiumpropyl-EDTA-Iron(11) Footprinting Analysis of DNA Binding Sites for Anthramycin and Related Drugs," *Biochemistry* 25 (1986): 1249–58.
14. J. Wei, J. Chen, J. Xu, L. Cao, H. Deng, W. Sheng, H. Zhang, and W. Cao, "Solution-Phase Perfluoroalkylation of C60 Leads to Efficient and Selective Synthesis of Bis-Perfluoroalkylated Fullerenes," *Journal of Fluorine Chemistry* 133 (2012): 146–54.
15. L. Liang and D. Astruc, "The Copper(I)-Catalyzed Alkyne-Azide Cycloaddition (CuAAC) "Click" Reaction and Its Applications," *Coordination Chemistry Reviews* 255, no. 23–24 (2011): 2933–45.
16. M. Meldal, "Polymer "Clicking" by CuAAC Reactions," *Macromolecular Rapid Communications* 29, no. 12–13 (2008): 1016–51.
17. V. D. Bock, H. Hiemstra, and J. H. van Maarseveen, "CuI -Catalyzed Alkyne–Azide "Click" Cycloadditions from a Mechanistic and Synthetic Perspective," *European Journal of Organic Chemistry* 2006, no. 1 (2006): 51–68.
18. M. Meldal and C. W. Tornøe, "Cu-Catalyzed Azide-Alkyne Cycloaddition," *Chemical Reviews* 108, no. 8 (2008): 2952–3015.
19. J. E. Hein and V. V. Fokin, "Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) and Beyond: New Reactivity of Copper(I) Acetylides," *Chemical Society Reviews* 39, no. 4 (2010): 1302–15. [20309487]
20. J. E. Moses and A. D. Moorhouse, "The Growing Applications of Click Chemistry," *Chemical Society Reviews* 36, no. 8 (2007): 1249–62.
21. X. L. Wang, K. Wan, and C. H. Zhou, "Synthesis of Novel Sulfanilamide-Derived 1,2,3-Triazoles and Their Evaluation for Antibacterial and Antifungal Activities," *European Journal of Medicinal Chemistry* 45, no. 10 (2010): 4631–9.
22. D. Dheer, V. Singh, and R. Shankar, "Medicinal Attributes of 1,2,3-Triazoles: Current Developments," *Bioorganic Chemistry* 71 (2017): 30–54.
23. P. H. Olesen, A. R. Sorensen, B. Urso, P. Kurtzhals, A. N. Bowler, U. Ehrbar, and B. F. Hansen, "Synthesis and In Vitro Characterization of 1-(4-Aminofurazan-3-yl)-5-Dialkylaminomethyl-1H-[1,2,3]Triazole-4-Carboxylic Acid Derivatives. A New Class of Selective GSK-3 Inhibitors," *Journal of Medicinal Chemistry* 46, no. 15 (2003): 3333–41.

24. E. K. Moltzen, H. Pedersen, K. P. Bogeso, E. Meier, K. Frederiksen, C. Sanchez, and K. L. Lembol, "Bioisosteres of Arecoline: 1,2,3,6-Tetrahydro-5-pyridyl-Substituted and 3-Piperidyl-Substituted Derivatives of Tetrazoles and 1,2,3-Triazoles. Synthesis and Muscarinic Activity," *Journal of Medicinal Chemistry* 37, no. 24 (1994): 4085–99.
25. A. Giraudo, J. Krall, B. Nielsen, T. E. Sorensen, K. T. Kongstad, B. Rolando, D. Boschi, B. Frolund, and M. L. Lolli, "4-Hydroxy-1,2,3-Triazole Moiety as Bioisostere of the Carboxylic Acid Function: A Novel Scaffold to Probe the Orthosteric γ-Aminobutyric Acid Receptor Binding Site," *European Journal of Medicinal Chemistry* 158 (2018): 311–21.
26. R. W. Carling, K. W. Moore, L. J. Street, D. Wild, C. Isted, P. D. Leeson, S. Thomos, D. O'Connor, R. M. Mckernan, K. Quirk, et al. "3-Phenyl-6-(2-Pyridyl)Methyloxy-1,2,4-Triazolo[3,4-a]Phthalazines and Analogues: High-Affinity Gamma-Aminobutyric acid-A Benzodiazepine Receptor Ligands with Alpha 2, Alpha 3, and Alpha 5-Subtype Binding Selectivity over Alpha 1," *Journal of Medicinal Chemistry* 47 (2004): 1087–822.
27. S. D. Hadiyal, N. D. Parmar, P. L. Kalavadiya, J. N. Lalpara, and H. S. Joshi, "Microwave-Assisted Three-Component Domino Synthesis of Polysubstituted 4H-Pyran Derivatives and Their Anticancer Activity," *Russian Journal of Organic Chemistry* 56, no. 4 (2020): 671–8.
28. N. D. Parmar, S. D. Hadiyal, V. H. Kapupara, and H. S. Joshi, "Microwave-Assisted Synthesis of (2-Butyl-5-Nitrobenzo[b]Furan-3-yl)-[4-(Substituted Ethynyl)Phenyl] Methanones," *Arkivoc* 2018, no. 7 (2018): 143–53.
29. M. R. Boyd and K. D. Paull, "Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen," *Drug Development Research* 34, no. 2 (1995): 91–109.
30. K. D. Paull, R. H. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman, and M. R. Boyd, "Display and Analysis of Patterns of Differential Activity of Drugs against Human Tumor Cell Lines: Development of Mean Graph and COMPARE Algorithm," *Journal of the National Cancer Institute* 81, no. 14 (1989): 1088–92.
31. M. Anne, S. Dominic Philip, S. Robert, S. Kenneth, V. David, H. Curtis, L. John, C. Paul, V. W. Anne, G. G. Marcia, et al. "Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines," *Journal of the National Cancer Institute* 83 (1991): 757–66.
32. C. M. Mann and J. L. Markham, "A New Method for Determining the Minimum Inhibitory Concentration of Essential Oils," *Journal of Applied Microbiology* 84, no. 4 (1998): 538–44.
33. T. E. A. Du and M. Rautenbach, "A Sensitive Standardised Micro-Gel Well Diffusion Assay for the Determination of Antimicrobial Activity," *Journal of Microbiological Methods* 42, no. 2 (2000): 159–65.
34. C. Valgas, S. M. De Souza, E. F. A. Smânia, and A. Smânia, "Screening Methods to Determine Antibacterial Activity of Natural Products," *Brazilian Journal of Microbiology* 38, no. 2 (2007): 369–80.