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# Synthesis of Novel 1,4-Disubstituted 1,2,3-Triazolo-Bosentan Derivatives – Evaluation of Antimicrobial, Anticancer activities and Molecular Docking

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K. Easwaramoorthi,[a,c] A. Jeya Rajendran,*[,a] K. Chennakesava Rao,[b,c] Y. Arun,[b] C. Balachandran,[d] P.T. Perumal,[b] Nobuhiko Emi,[d] S.M. Mahalingam[e] V.Duraipandiyan[f,g] N.A. Al-Dhabi[g]

Novel 1,4-disubstituted 1,2,3-triazolo bosentan derivatives **1a-n** from bosentan **2** were accomplished in good yields by sequential chlorination, azidation followed by Cu(I) catalyzed 1,3-dipolar cycloaddition. All obtained compounds **1a-n** were evaluated for their antimicrobial, *in vitro* anticancer activities and *in silico* docking studies. Among all tested compounds **1e-f** and **1h-j** have shown better antimicrobial activities against tested bacteria and fungi. When subjected to anticancer activity, compounds **1g-j** and **1n** have shown significant activities against both A549 and SKOV-3 cell lines with IC50 values at 7.81 μg/mL and among them compound **1i** has exhibited very potent activity. Beside no toxicity was calculated up to 2 mg/mL in Vero cells. *In silico* studies were conducted to know the possible bonding modes of **1a-n** with target receptors namely DNA Topoisomerase IV (4 EMV) and Anaplastic Lymphoma Kinase (2XP2). Among them, compounds **1e** and **1h** have shown maximum binding energies with 4EMV and 2XP2 receptors, respectively which also exhibited good antimicrobial and potent anticancer activities.

## Introduction

As per WHO, multidrug-resistant organisms (MDRO) are an increasing threat to the human life as well as the animal kingdom.[1-5] Microorganisms gain resistance by mutation of their DNA and tolerate the effect of standard antimicrobial drugs resulting in uncured diseases/infections. Cancer is another major threat to human life. When in the early years of a person's life, normal body cells divide faster to allow him/her to grow well. The division of cell rate decreases when the person becomes an adult and it happens only to replace exhausted cells or to heal injuries. Abnormal growth of cells results to form cancers which are very difficult to cure and end with death in most cases. Most of the anticancer drugs have side effects on normal cells and result in some abnormalities in health condition. Hence, this situation stresses the recurrent development of new drugs that are more efficient and less expensive[6] than the existing drugs.

Endothelin A ($ET_A$) and B ($ET_B$) receptors, have been implicated in the development of several cancers through activation of pathways involved in cell proliferation, migration, invasion, osteogenesis and angiogenesis.[7] Targeting ET receptor and its antagonism constitute as an attractive and challenging approach for cancer therapy.[8-9] A large number of ET antagonists have been developed and studied in clinical trials for chronic heart failure, hypertension, cancer and fibrosis.[10-13] Bosentan, the dual $ET_A$/$ET_B$ receptor antagonist is approved for treatments of pulmonary arterial hypertention[14-16] and used in combination chemotherapy with cisplatin[17] and paclitaxel for ovarian cancer.[18] The development of potent bosentan derivative as specific, selective and dual ET receptor antagonist represents an effective treatment for cancer as a mono drug. Structural modification of bosentan may lead to increase in the selective dual activity with ET receptor and may cause less or no hepatotoxic (damage to liver – side effect of Bosentan) and anemia.[19-20] The current research activity focuses on the preparation of various 1,4-disubstituted 1,2,3-triazoles linked with the pyrimidine ring of bosentan to give triazolo-bosentan derivatives in order to test their anticancer, antimicrobial activities and carry out molecular docking studies.

Generally, having two is better than one and the same concept is applicable to hybrid drugs which can have enhanced biological activity and can address the issues on drug resistance.[21-23] Many hybrid drugs are known with more advantages, as they can potentially overcome the pharmacokinetic drawbacks than their precursors and conventional drugs.[24-26] These can be prepared by adjoining to

[a] Department of Chemistry, Loyola College, Chennai-600034, India, Phone: +91 944-411-6528, Email: jeyaadmaterial@gmail.com
[b] Organic Chemistry Division, CSIR-CLRI, Chennai-600020, India
[c] R&D Centre, Malladi Drugs & Pharmaceuticals Ltd., Chennai-600124, India.
[d] Department of Hematology, Fujita Health University, 1-98, Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470-1192, Japan.
[e] Centre for Drug Discovery and Department of Chemistry, Purdue University, Indiana
[f] Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai-600034, India
[g] Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saudi University,P.O.Box.2455, Riyadh-11451, Saudi Arabia.
†Electronic Supplementary Information (ESI) available: IR, NMR, Mass and Docking.

two different drugs by a covalent bond or by an adequate fusion.[27] Hybrid drugs such as NOSH-Aspirin[28], Artemisinin-quinine[29], reversed Chloroquine[30] are well known (Figure 1).

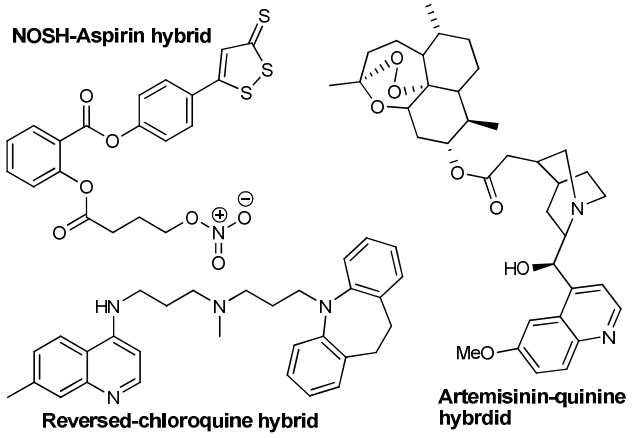



**Fig. 1**: Hybrid drugs

Hybrid compounds of pyrimidines which are associated with other heterocycles exhibited good biological activities.[31] Presently, the interest in developing new hybrid molecules from pyrimidine derivatives is increasing with vast applications in medicinal chemistry.[32-37] Click chemistry is a brilliant concept in organic chemistry which involves synthesizing complex molecules in high yields with simple isolation techniques.[38-39] Various 1,4-disubstituted 1,2,3-triazole derivatives have been reported in literature using this well known click chemistry approach by Cu(I) catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC)[40-45] which have broad applications in medicinal chemistry.[46-49]

## Results and discussion

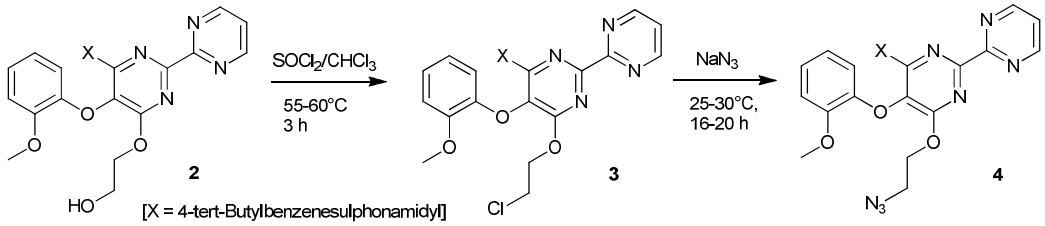
### Chemistry

The key intermediate azide **4** was prepared by heating bosentan **2** with thionyl chloride in chloroform followed by treating with sodium azide in *N,N*-dimethylacetamide (DMA) (Scheme 1**)**. The targeted new bis-pyrimidinyl linked 1,2,3-triazolo-bosentan derivatives **1a-n** were prepared via one pot synthesis by reacting azide **4** with various terminal alkynes **5a'-k'** and **5l-n** in the presence of CuI catalyst and *N, N*-diisopropylethylamine (DIPEA) in aqueous n-butanol medium at ambient temperature using a regular click chemistry approach (Scheme 2). Various conditions have been tried to optimize the method (Table 1). Terminal alkynes **5a'-f'** were obtained *in situ* by heating their corresponding amines **5a-f** with propargyl chloride in the presence of a base (Scheme 2). Terminal alkynes **5g'-k'** were also made *in situ* by converting their corresponding acids **5g-k** to acid chlorides followed by treating with propargyl alcohol (Scheme 2). Terminal alkynes **5l-n** were procured commercially and used as such. About 78-95% of overall yields (Scheme 4 and Table 2) were obtained when the optimized reaction conditions mentioned in this article were followed. The compounds **1a-n** were obtained as pale brown to brown amorphous solids and further purification also resulted in amorphous solids since single crystal XRD studies were not performed.

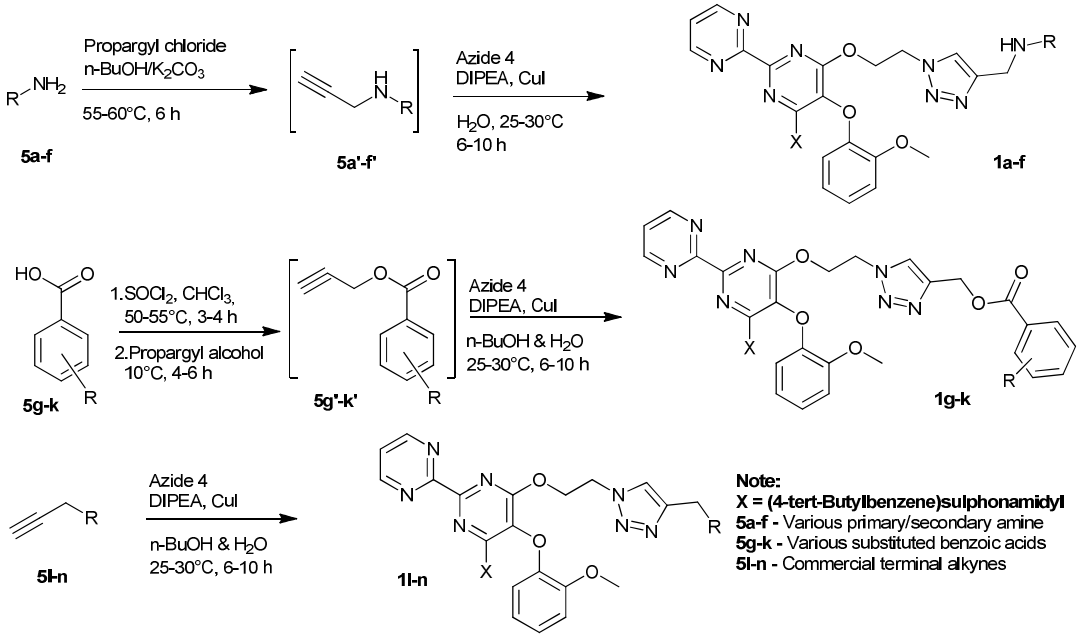
### Optimization of reaction conditions

Various solvents, bases, catalysts, reaction temperature and time have been explored to optimize the reaction conditions during this research work (Table 1) using Scheme 3 as a model experiment. During the study, it was noticed that better results were obtained when CuI (10 mol%), DIPEA and n-BuOH/Water mixture were used at room temperature for about 10 h (Table 1, entry 6). Usage of excess CuI did not improve the yield (Table 1, entry 5, 14 & 15) and usage of other copper salts was resulted in very poor conversions (Table 1, entry 2-3). It was understood that reaction can proceed in other polar solvents but had not gone to completion (Table 1, entries 6-11 & 12). The reaction did not proceed when other bases such as triethylamine and potassium hydroxide were used instead of DIPEA (Table 1, entries 12-15).

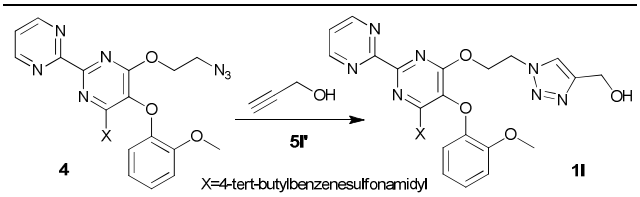
The structures of all novel 1,2,3-triazole **1a-n** compounds were elucidated with the help of IR, $^{1}$H-NMR, $^{13}$C-NMR and Mass spectra as exemplified for compound **1e.** Characteristic IR bands of **1e**: The broad band at 3422 cm$^{-1}$ represents the –NH stretching. The medium band at 2963 cm$^{-1}$ indicates the aliphatic stretching. The sharp bands that appear at 1674, 1605 & 1564 cm$^{-1}$ show the presence of benzenoid rings. The band present at 1393 cm$^{-1}$ confirms the presence of S=O stretching. The bands at 1252, 1219 & 1173 cm$^{-1}$ support the presence of –C–N and –C–O stretching. The characteristic bands at 864, 762 & 694 cm$^{-1}$ represent the existence of *para*- & *ortho*- disubstituted and monosubstituted aromatic rings respectively. The signals of $^{1}$H & $^{13}$C -NMR of compound **1e** are explained in Figure 1. A distinguishing peak observed at *m/z*: 748 in the mass spectrum of **1e** corresponds to the protonated molecular ion $[M+H]^{+}$.



**Scheme 1:** Synthesis of azide **4** from Bosentan **2**




**Scheme 2:** Overview of synthesis of novel 1,4-disubstituted 1,2,3-triazoles **1a-n**




**Scheme 3**: Synthesis of **1l** – Reaction optimization

**Table 1:** Optimization of reaction conditions

| Entry | Base | Catalyst (mol%) | Solvent | Temp (°C) | Time (h) | Yield (%)[a] |
|---|---|---|---|---|---|---|
| 1 | DIPEA | CuI (10) | n-BuOH | 25-30 | 10 | 75 |
| 2 | DIPEA | CuBr (10) | n-BuOH | 25-30 | 10 | 15 |
| 3 | DIPEA | CuCl (10) | n-BuOH | 25-30 | 10 | trace |
| 4 | DIPEA | CuI (5) | n-BuOH | 25-30 | 10 | 46 |
| 5 | DIPEA | CuI (20) | n-BuOH | 25-30 | 10 | 93 |
| 6 | DIPEA | CuI (10) | $H_2O$ | 25-30 | 10 | 48 |
| 7 | DIPEA | CuI (10) | n-BuOH/ $H_2O$(1:1) | 25-30 | 10 | 95 |
| 8 | DIPEA | CuI (10) | EtOH | 25-30 | 10 | trace |
| 9 | DIPEA | CuI (10) | EtOH | 45-50 | 20 | 15 |
| 10 | DIPEA | CuI (10) | EtOH/ $H_2O$(1:1) | 45-50 | 20 | 27 |
| 11 | DIPEA | CuI (10) | THF | 25-30 | 20 | 60 |
| 12 | TEA | CuI (10) | n-BuOH | 25-30 | 10 | 31 |
| 13 | TEA | CuI (10) | THF | 25-30 | 10 | 46 |
| 14 | KOH | CuI (20) | n-BuOH | 25-30 | 10 | trace |
| 15 | KOH | CuI (20) | n-BuOH | 50-55 | 20 | trace |

DIPEA–diisopropylethylamine, TEA–triethylamine, [a]isolated yields

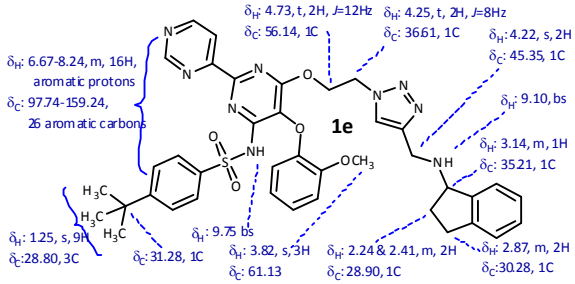


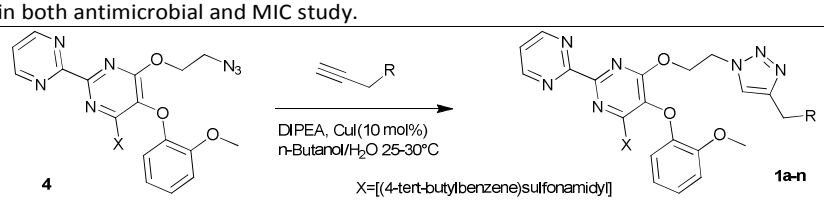

**Fig. 2**: Characterization of **1e** using NMR spectroscopy

## Pharmacology

### Antimicrobial activity

The synthesized novel bis-pyrimidinyl linked 1,2,3-triazolo-bosentan derivatives **1a-n** were screened for antimicrobial activity[50] using the well method[51] at 1000 $\mu$g/well against eleven bacteria and two fungi **(Table 3)**. Minimum inhibitory concentration (MIC) studies of the synthesized compounds **1a-n** were performed according to the standard reference method for bacteria, filamentous fungi[52] and yeasts[53] (NCCLS/CLSI, 2002). The MIC values of the synthesized compounds **1a-n** were calculated (**Table 3)**. Standard antimicrobial drugs, streptomycin, ciprofloxacin and

ketoconazole were used as positive controls against bacteria and fungi respectively in both antimicrobial and MIC study.




**Scheme 4:** One pot synthesis of 1,4-disubstituted 1,2,3-triazoles **1a-n** from azide **4** using click chemistry

**Table 2**: Novel 1,4-disubstituted 1,2,3-triazoles from bosentan

| Compound | Terminyl alkynes prepared from | R | m.p (°C)* | Yield (%)# |
|---|---|---|---|---|
| **1a** | Ephedrine | [N-methyl-(1-hydroxy-1-phenyl)-2-propylamino] | 122-125 | 88 |
| **1b** | Norephedrine | [(1-hydroxy-1-phenyl)-2-propylamino] | 140-143 | 78 |
| **1c** | Selegiline | [1-phenyl-2-propylamino] | 99-102 | 90 |
| **1d** | Phenylephrine | [N-methyl-2-(3-hydroxyphenyl)-2-hydroxyethylamino] | 148-150 | 90 |
| **1e** | 1-Aminoindane | [2,3-dihydro-1H-inden-1-amino] | 102-105 | 88 |
| **1f** | Noralfentanil | [4-(methoxymethyl)-4-(N-phenyl-N-propionamide)piperidino] | 180-181 | 91 |
| **1g** | Benzoic acid | [benzoyloxy] | 95-98 | 92 |
| **1h** | 2-Bromo-5-methoxybenzoic acid | [2-bromo-5-methoxybenzoyloxy] | 160-164 | 84 |
| **1i** | 4-Nitrobenzoic acid | [4-nitrobenzoyloxy] | 168-171 | 88 |
| **1j** | Veratroic acid | [3,4-dimethoxybenzoyloxy] | 128-132 | 90 |
| **1k** | 3-Methylbenzoic acid | [4-methylbenzoyloxy] | 140-144 | 87 |
| **1l** | Propargyl alcohol€ | [hydroxymethyl] | 168-170 | 95 |
| **1m** | 1-Hexyne€ | [n-butyl] | 101-103 | 89 |
| **1n** | 1-Ethynyl-1-cyclohexanol€ | [(1-hydroxy)cyclohexyl] | 105-107 | 87 |

* uncorrected melting points, # obtained yields without any purification, € obtained from commercial sources and used as such

The incurred results disclosed good antimicrobial activity of the synthesized compounds **1a**-**n** when compared with the standard antimicrobial drugs used in this study. *P. aeruginosa* has emerged as one of the most problematic Gram-negative pathogen, with an alarmingly high antibiotic resistance rate. Even with the most effective antibiotics against this pathogen, namely carbapenems (imipenem and meropenem), the resistance rate was found to be 15-20.4% amongst *aeruginosa* strains.[54] Our current study showed that the synthesized compounds were active against *P. aeruginosa.* Compounds, **1e**-**f** & **h-j** have shown better activity when compared with others against tested bacteria and fungi. Best MIC values have been observed for compounds **1h-j** amongst others**.** The ground for this better activity in **1h** and **1j** may be due to the presence of methoxy substitutions of benzoic acid which can increase the *in vitro* activity against gram positive when compared to other compounds.[55-56] The reason for the best activity of **1i** may be due to the presence of *para*-nitro substitution in benzoic acid.

**Table 3:** Antimicrobial activity of synthesized compounds **1a-n** using the well method (zone of inhibition in mm) (1000 µg/well).

| Organism | Compounds | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1a** | **1b** | **1c** | **1d** | **1e** | **1f** | **1g** | **1h** | **1i** | **1j** | **1k** | **1l** | **1m** | **1n** | **C** |
| **Gram positive bacteria** | | | | | | | | | | | | | | | |
| *Bacillus subtilis* | 16 | 18 | - | 14 | 12 | 14 | 18 | 20 | 19 | 19 | 14 | - | 14 | 18 | 24 |
| *Micrococcus luteus* | 15 | 12 | - | 14 | 20 | 12 | 17 | 15 | 21 | 16 | 12 | - | 14 | 18 | 26 |
| *Staphylococcus aureus* | 13 | - | - | 12 | 13 | 13 | 12 | 15 | 11 | 14 | 10 | - | 17 | 16 | 14 |
| *Staphylococcus epidermidis* | 14 | 16 | - | 10 | 14 | 14 | 22 | 21 | 24 | 19 | 14 | - | 15 | 20 | 26 |
| **Gram negative bacteria** | | | | | | | | | | | | | | | |
| *Klebsiellapneumoniae* | 13 | 14 | - | 15 | 12 | 17 | 21 | 18 | 19 | 18 | 10 | - | 13 | 16 | 20 |
| *Salmonella typhimurium* | 12 | 13 | - | - | 16 | 16 | 14 | - | - | - | - | - | - | 14 | 24 |
| *Proteus vulgaris* | 13 | 16 | - | 14 | 18 | 13 | 17 | 20 | 15 | 20 | 10 | - | 16 | 18 | 30 |
| *Shigella flexneri* | 15 | - | - | 15 | 19 | 12 | 18 | 25 | 21 | 20 | 12 | - | 18 | 19 | 30 |
| *Enterobacter aerogenes* | 16 | 15 | - | 14 | 15 | 16 | 20 | 18 | 18 | 17 | 10 | - | 16 | 17 | 22 |
| *Pseudomonas aeruginosa* | 14 | 18 | - | 15 | 16 | 13 | 20 | 22 | 21 | 18 | 13 | - | 16 | 18 | 30 |
| *Staphylococcus aureus* MRSA | 15 | 12 | - | 13 | 18 | 10 | 23 | 22 | 19 | 17 | 16 | - | 14 | 16 | 30 |
| **Fungi** | | | | | | | | | | | | - | | | |
| *Candida albicans* | 16 | 15 | - | - | - | - | 15 | - | 14 | 16 | - | - | 10 | 14 | 28 |
| *Malassesia pachydermatis* | - | 13 | - | - | - | - | - | - | - | 12 | - | - | 13 | - | 26 |

C: Streptomycin - standard antibacterial agent; C: Ketoconazole - standard antifungal agent

**Table 4:** Minimum inhibitory concentration (MIC) of synthesized compounds **1a-n**

| Organism | Compounds | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 1b | 1d | 1e | 1f | 1g | 1h | 1i | 1j | 1k | 1m | 1n | C-1 | C-2 |
| **Gram positive bacteria** | | | | | | | | | | | | | | |
| *Bacillus subtilis* | 62.5 | 62.5 | 125 | 62.5 | 125 | 62.5 | 31.25 | 31.25 | 62.5 | 125 | 250 | 125 | 25 | <0.78 |
| *Micrococcus luteus* | 250 | 500 | 250 | 62.5 | 125 | 500 | 62.5 | 62.5 | 125 | 500 | 250 | 125 | 6.25 | >100 |
| *Staphylococcus aureus* | 250 | - | 500 | 250 | 250 | 250 | 250 | 125 | 250 | 500 | 125 | 125 | 6.25 | <0.78 |
| *Staphylococcus epidermidis* | 250 | 125 | 500 | 250 | 62.5 | 250 | 62.5 | 31.2 | 62.5 | 250 | 250 | 62.5 | 25 | 6.25 |
| **Gram negative bacteria** | | | | | | | | | | | | | | |
| *Klebsiella pneumonia* | 250 | 250 | 250 | 125 | 62.5 | 125 | 62.5 | 125 | 62.5 | 500 | 250 | 125 | 6.25 | 6.25 |
| *Salmonella typhimurium* | 500 | 250 | - | 125 | 250 | 125 | 125 | - | - | - | - | 250 | 30 | >100 |
| *Proteus vulgaris* | 250 | 125 | 250 | 125 | 125 | 250 | 125 | 250 | 62.5 | 500 | 125 | 125 | 6.25 | 6.25 |
| *Shigella flexneri* | 250 | - | 250 | 125 | 125 | 500 | 125 | 62.5 | 62.5 | 500 | 125 | 125 | 6.25 | <0.78 |
| *Enterobacter aerogenes* | 125 | 250 | 250 | 250 | 125 | 125 | 62.5 | 62.5 | 125 | 500 | 125 | 125 | 25 | <0.78 |
| *Pseudomonas aeruginosa* | 125 | 62.5 | 125 | 62.5 | 125 | 31.25 | 31.25 | 31.25 | 62.5 | 250 | 125 | 125 | 25 | 6.25 |
| *Staphylococcus aureus* MRSA | 250 | 500 | 500 | 125 | 31.2 | 500 | 31.2 | 125 | 125 | 250 | 250 | 125 | 6.25 | <0.78 |

C-1: Streptomycin and C-2: Ciprofloxacin - standard antibacterial agents

## Anticancer activity

Based on antimicrobial results, compounds **1b** and **1e-n** were taken selectively and screened for anticancer activity against A549 and SKOV-3 cancer cell lines.[57] All the tested compounds showed good cytotoxicity activity against A549 and SKOV-3 cell lines, however, some of the synthesized compounds showed prominent cytotoxicity activity *in vitro* against A549 and SKOV-3 cell lines. The anticancer activity against A549 and SKOV-3 cell lines were observed in 250 to 7.81 µg/mL concentrations (Table 5,6 and Figures 3,4). The results showed that compounds **1e-j** & **1l** have shown significant activity against A549 cell line when compared to other compounds. In particular, **Ig,i-j & 1l** have exhibited very potent cytotoxicity activity against A549 cell line with IC50 values at 7.81 µg/mL. Interestingly significant activity against SKOV-3 cell lines was also noticed for compounds **1e-j** & **1l-n** when compared with other compounds. Among the tested compounds **1g,i-j** & **1n** showed potent cytotoxicity activity against SKVO-3 cell line with IC50 values at 7.81 µg/mL. All concentrations used in the experiment decreased the cell viability significantly ($P<0.05$) in a concentration-dependent manner. Interestingly, significant anticancer results were seen in triazolo-bosentan derivatives prepared by using aromatic carboxylic acids **5g-j** except **1k.** Compound **1b**, prepared from an amine **5b** was found to be least active when compared to others. Compound **1i** has shown best activity for both cell lines and the reason might be the presence of an electron withdrawing group ($-NO_2$) at the *para*- position of benzoic acid **5i**. Triazolo-bosentans obtained from aliphatic alkynes **1l-n** also showed good activity. Toxicity study was tested against Vero cells. Interestingly triazolo-bosentan derivatives of **1e-j, 1l,** and **1n** showed no toxicity up to 2 mg/mL.

**Table 5**. Anticancer activity of synthesized compounds against A549 cancer cell line

| Concentration (µg/mL) | | Compound | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1b | 1e | 1f | 1g | 1h | 1i | 1j | 1k | 1l | 1m | 1n |
| 250 | % | 31.3 | 89.2 | 91.5 | 96.3 | 94.7 | 96.2 | 94.2 | 47.2 | 95.6 | 19 | 93.7 |
| | Mean | 1.345 | 0.211 | 0.166 | 0.072 | 0.103± | 0.074 | 0.114± | 1.033 | 0.087 | 1.586 | 0.123 |
| | ±S.D. | ±0.00325 | ±0.00839 | ±0.00712 | ±0.00337 | 0.00441 | ±0.00118 | 0.00890 | ±0.00881 | ±0.00879 | ±0.00557 | ±0.00997 |
| 125 | % | 27 | 84.9 | 62.5 | 95.8 | 87 | 95.5 | 90.9 | 35.6 | 90.8 | 16.7 | 80.9 |
| | Mean | 1.429 | 0.295 | 0.735 | 0.082 | 0.255 | 0.088 | 0.179± | 1.261 | 0.18 | 1.631 | 0.123 |
| | ±S.D. | ±0.00412 | ±0.00559 | ±0.00660 | ±0.00559 | ±0.00578 | ±0.00229 | 0.00773 | ±0.00836 | ±0.00706 | ±0.00967 | ±0.00997 |
| 62.5 | % | 24.9 | 51.2 | 18.2 | 82.7 | 81.5 | 95.3 | 86.3 | 25.6 | 80.6 | 14.9 | 56.1 |
| | Mean | 1.47 | 0.955± | 1.602 | 0.338 | 0.363 | 0.091 | 0.268± | 1.456 | 0.38 | 1.666 | 0.859 |
| | ±S.D. | ±0.00522 | 0.00436 | ±0.00578 | ±0.00631 | ±0.00669 | ±0.00397 | 0.00559 | ±0.00771 | ±0.00756 | ±0.00805 | ±0.00361 |
| 31.25 | % | 16.4 | 42.9 | 13.5 | 71.9 | 60.6 | 79.9 | 75 | 14.5 | 77.9 | 10.2 | 18.6 |
| | Mean | 1.636 | 1.118± | 1.694 | 0.55 | 0.771 | 0.393 | 0.489± | 1.675 | 0.432 | 1.759 | 1.594 |
| | ±S.D. | ±0.00632 | 0.00947 | ±0.00309 | ±0.00779 | ±0.00330 | ±0.00433 | 0.00397 | ±0.00743 | ±0.00801 | ±0.00598 | ±0.00772 |
| 15.63 | % | 13.1 | 30.9 | 11.9 | 68.1 | 51.2 | 75 | 71.6 | 11.1 | 66.1 | 8.2 | 15.5 |
| | Mean | 1.701 | 1.352± | 1.725 | 0.625 | 0.956 | 0.489 | 0.556± | 1.741 | 0.663 | 1.797 | 1.654 |
| | ±S.D. | ±0.00203 | 0.00871 | ±0.00399 | ±0.00597 | ±0.00449 | ±0.00507 | 0.00227 | ±0.00491 | ±0.00699 | ±0.00671 | ±0.00589 |
| 7.81 | % | 5.2 | 26.7 | 8.6 | 62 | 30.5 | 68.2 | 57.8 | 8.1 | 54.1 | 6.7 | 9.6 |
| | Mean | 1.856 | 1.436± | 1.789 | 0.745 | 1.361 | 0.623 | 0.826± | 1.799 | 0.899 | 1.826 | 1.77 |
| | ±S.D. | ±0.00654 | 0.00856 | ±0.00580 | ±0.00888 | ±0.00379 | ±0.00409 | 0.01025 | ±0.00513 | ±0.00712 | ±0.00334 | ±0.00665 |

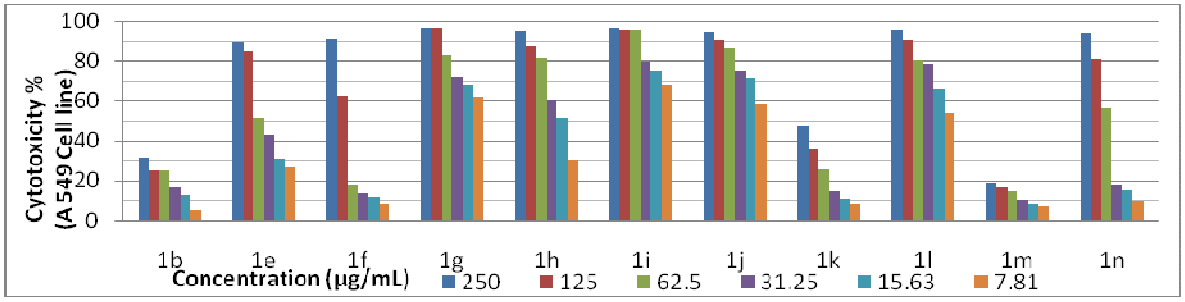



**Fig. 3:** Comparison of anticancer activity of synthesized compounds against A 549 cancer cell line
(Note: No toxicity was observed up to 2mg/mL in Vero cells)

**Table 6**: Anticancer activity of synthesized compounds against SKOV-3 cancer cell line

| Concentration (µg/mL) | | Compound 1b | 1e | 1f | 1g | 1h | 1i | 1j | 1lk | 1l | 1m | 1n |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 250 | % | 48.9 | 71.2 | 81.2 | 94.3 | 94 | 94.7 | 92.6 | 39.9 | 95.3 | 84.7 | 91.6 |
| | Mean | 0.653 | 0.368 | 0.24 | 0.073 | 0.077 | 0.06 | 0.095 | 0.768 | 0.06 | 0.195 | 0.107 |
| | S.D. | ±0.00338 | ±0.00443 | ±0.00556 | ±0.00702 | ±0.00507 | 8±0.00560 | ±0.00363 | ±0.00338 | ±0.00447 | ±0.00337 | ±0.00447 |
| 125 | % | 40.5 | 54 | 64.9 | 87.2 | 92.2 | 93.7 | 83.6 | 34.9 | 93.9 | 77.7 | 81 |
| | Mean | 0.76 | 0.588 | 0.448 | 0.164 | 0.1 | 0.08 | 0.21 | 0.831 | 0.078 | 0.285 | 0.242 |
| | S.D. | ±0.00561 | ±0.00881 | ±0.00773 | ±0.00564 | ±0.00812 | ±0.00609 | ±0.00569 | ±0.00501 | ±0.00510 | ±0.00429 | ±0.00366 |
| 62.5 | % | 34.1 | 42 | 40.4 | 80.3 | 89.8 | 91.6 | 81.8 | 23.4 | 63.8 | 65.3 | 86.3 |
| | Mean | 0.841 | 0.741 | 0.761 | 0.252 | 0.13 | 0.107 | 0.232 | 0.978 | 0.462 | 0.443 | 0.175 |
| | ±S.D. | ±0.00201 | ±0.00779 | ±0.00501 | ±0.00328 | ±0.00328 | ±0.00544 | ±0.00438 | ±0.00588 | ±0.00553 | ±0.00577 | ±0.00228 |
| 31.25 | % | 31.6 | 40.1 | 30.1 | 69.5 | 65.8 | 85.2 | 77.6 | 16.2 | 54.9 | 50.1 | 80.7 |
| | Mean | 0.874 | 0.765 | 0.893 | 0.389 | 0.437 | 0.189 | 0.286 | 1.07 | 0.576 | 0.637 | 0.246 |
| | S.D. | ±0.00198 | ±0.00278 | ±0.00337 | ±0.00209 | ±0.00449 | ±0.00224 | ±0.00371 | ±0.00579 | ±0.00438 | ±0.00804 | ±0.00509 |
| 15.63 | % | 24.6 | 28.6 | 22 | 63.6 | 57.1 | 81.9 | 72.1 | 9.2 | 51.1 | 31.2 | 75.3 |
| | Mean | 0.963 | 0.912 | 0.996 | 0.465 | 0.548 | 0.231 | 0.356 | 1.159 | 0.625 | 0.878 | 0.315 |
| | ±S.D. | ±0.00854 | ±0.00900 | ±0.00886 | ±0.00667 | ±0.00579 | ±0.00697 | ±0.00399 | ±0.00416 | ±0.00688 | ±0.00331 | ±0.00708 |
| 7.81 | % | 6 | 22.7 | 9.5 | 60.1 | 50.3 | 76.4 | 63.4 | 3.2 | 33.7 | 22.2 | 63.3 |
| | Mean | 1.201 | 0.987 | 1.156 | 0.51 | 0.63 | 0.301 | 0.468 | 1.236 | 0.847 | 0.993 | 0.469 |
| | S.D. | ±0.00337 | ±0.00458 | ±0.00927 | ±0.00345 | 5±0.00670 | ±0.00555 | ±0.00608 | ±0.00399 | ±0.00701 | ±0.00209 | ±0.00884 |

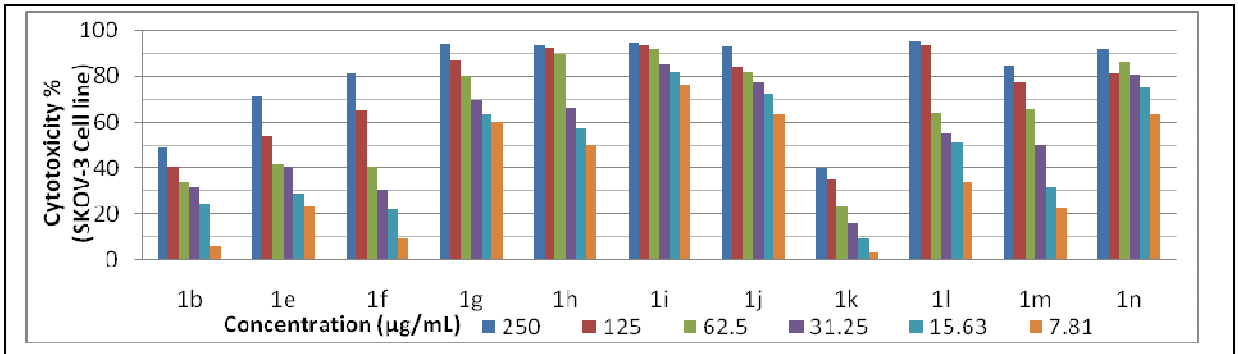



**Fig. 4:** Comparison of anticancer activity of synthesized compounds against SKOV-3 cancer cell line

***In silico* studies**

Molecular docking is a useful instrument to acquire knowledge on protein-ligand interaction which is very significant in drug discovery.[58] All the synthesized pyrimidinyl linked 1,2,3-triazolo-bosentan derivatives **1a-n** were subjected to molecular docking studies with DNA topoisomerase IV (PDB ID: 4EMV) and Anaplastic Lymphoma Kinase (ALK) (PDB ID: 2XP2) using the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program[59-60] to investigate the potential binding mode of inhibitors. The DNA Topoisomerase IV receptor is required for maintenance of proper DNA topology during transcription and replication in bacteria.[61] Anaplastic Lymphoma Kinase (ALK) is a Tyrosine kinase receptor. Due to its critical role of aberrant signalling in cancer, it is an attractive oncology target for therapeutic

intervention.[62] The obtained free energy of binding (FEB) was computed for all the synthesized compounds **1a-n (Table 7)**.

**Table 7** Free energy of binding (FEB) of **1a-n**

| Compound | Free energy of binding (*kcal/mol*)[a] | |
|---|---|---|
| | DNA Topoisomerase IV (4EMV) | Anaplastic Lymphoma Kinase (2XP2) |
| 1a | -6.95 | NC |
| 1b | -5.23 | -8.54 |
| 1c | -6.58 | NC |
| 1d | -5.53 | NC |
| 1e | -9.29 | -8.66 |
| 1f | -7.97 | -8.01 |
| 1g | -8.40 | -8.32 |
| 1h | -6.71 | -9.55 |
| 1i | -7.09 | -7.94 |
| 1j | -8.13 | -6.70 |
| 1k | -7.04 | -8.70 |
| 1l | -6.08 | -8.42 |
| 1m | -7.44 | -8.78 |
| 1n | -7.28 | -8.28 |
| Inhibitor | -9.80 | -8.42 |

[a]Calculated by Autodock, NC-not calculated, Inhibitor: Co-crystallized inhibitor with protein

Molecular docking outcomes of synthesized compounds **1a-n** with 4EMV receptor established that all the docked compounds **1a-n** bind efficiently with the receptor and exhibit free energy of binding value from –5.23 to –9.29 *kcal/mol*. Interestingly, among all the compounds docked, compound **1e** exhibits very high binding with 4EMV receptor and forms five polar interactions with three amino acids, namely ASP-78, GLY-82 and ARG-140, resulting in the binding energy of –9.29 *kcal/mol*. In the compound **1e**, N-H attached with triazole interacts with the ASP-78 and forms two polar interactions with bond lengths of 1.7 and 3.3 Å respectively. Likewise, two nitrogens of triazole interact with the GLY-82 and form two polar interactions with bond lengths of 3.2 and 3.5 Å respectively. Aside from this, pyrimidine nitrogen forms a polar interaction with the ARG-140 with a bond length of 2.9 Å (**Fig. 5**).

Docking of synthesized compounds with 2XP2 revealed that, compounds efficiently bind with the active site of 2XP2 receptor and exhibits free energies of binding from -6.70 to -9.55 kcal/mol. Compounds interact with the active site amino acids of 2XP2 namely ARG-1120, LEU-1122, GLY-1123, VAL-1130, GLU-1132, ALA-1148, LYS-1150, LEU-1196, GLU-1197, LEU-1198, MET-1199, ALA-1200, GLY-1201, GLY-1202, ASP-1203, SER-1206, PHE-1207, GLU-1210, ARG-1253, ASN-1254, CYS-1255, LEU-1256, GLY-1269 and ASP-1270.Among all the compounds docked, compound **1h** exhibited very high binding with 2XP2 receptor and formed seven polar interactions with five amino acids and resulted in a binding energy of -9.55 kcal/mol. In the compound **1h**, nitrogens of two pyrimidines interact with C=O of LEU-1122 and ASP-1203 to form three polar interactions with bond lengths of 2.7, 3.2 and 3.4 Å. In addition, nitrogen and oxygen of triazole and phenoxy groups

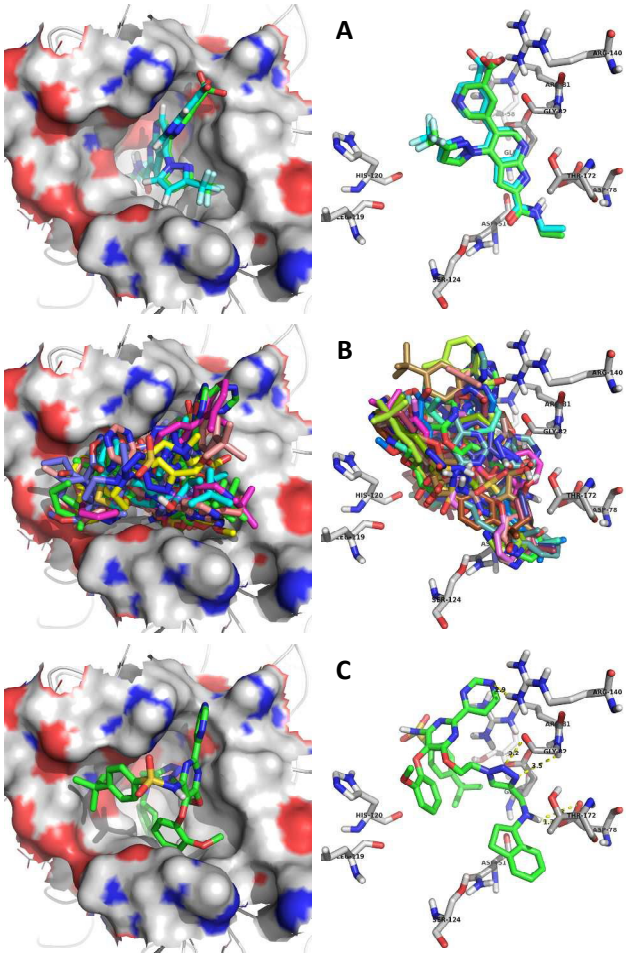



**Fig. 5:** Docking with 4EMV receptor (A) Method validation using crystallised and docked ligand; (B) Docking mode of all the compounds; (c) Docking mode of the most binding energy compound **1e**.

interact with C=O and N-H of MET-1199 and LYS-1150 to form two polar interactions of 2.0 and 3.4 Å respectively. Also, N-H and S=O interact with C=O of GLY-1123 to form two polar interactions in 2.0 and 3.4 Å respectively. Binding interaction with the 2XP2 receptor is shown in **Fig.6**.

## Conclusions

In summary, we have described facile one pot synthesis of novel bis-pyrimidinyl linked 1,4-disubstituted 1,2,3-triazoles **1a-n** (triazolo-bosentans) with good yields from bosentan **2** as a potent hybrid drugs with dual biological activities (antimicrobial and anticancer) and rationalized with respective proteins using docking study. During this work good antimicrobial activities were observed for compounds **1e-f** & **1h-j** against tested bacteria and fungi, amongst which, **1h** and **1j** exhibited very good activity. *In vitro c*ytotoxicity (lung and ovarian) studies against A549 and SKOV-3 cell lines revealed

prominent cytotoxic activity of compounds **1e-n**. Among all triazolo-bosentans screened, **(1g,i-j)** and **(1l,e-j,l-n)** showed very significant *in vitro* cytotoxicity against A549 and SKOV-3 cell lines respectively with IC50 value at 7.81 µg/mL. Active compounds **1e-j, 1l** and **1n** were tested against Vero cells and no toxicity was observed up to 2 mg/mL. When correlated the structure and activity, it was concluded that antimicrobial and anticancer activities were enhanced by the methoxy substitutions of benzoic acid ring. Among the tested compounds, best anticancer activity was observed for *p*-nitro substituted triazolo-bosentan **1i** which also exhibited good antimicrobial activity. Free energies of binding were calculated for the synthesized compounds against 4EMV and 2XP2 receptors. Among them, **1e** has shown a better binding energy (-9.29 *kcal/mol*) against 4EMV receptor with five polar interactions by three amino acids and **1h** has shown better binding energy (-9.55 *kcal/mol*) against 2PX2 receptor with seven polar interactions by five amino acids. Based on significant anticancer results, this class of compounds can be considered as a good starting point of development of bosentan **2** based dual endothelin receptor (ET) antagonists for efficient mono therapy for cancer.

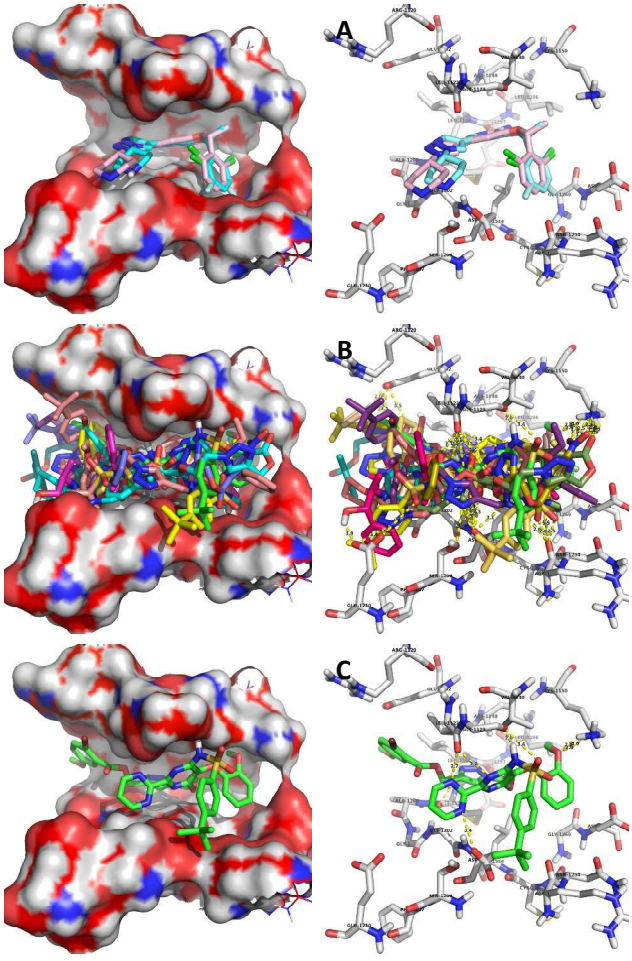



**Fig. 6:** Docking with 2XP2 receptor (A) Method validation using crystallised and docked ligand; (B) Docking mode of all the compounds; (C) Docking mode of the most binding energy compound **1h.**

## Acknowledgements

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## Experimental

### Chemistry

Melting points were measured using a Veego melting point apparatus model VMP-PM. Thin layer chromatography (TLC) was carried out using pre-coated Merck TLC Silicagel 60 F254 and spots were detected using Ultra-Violet light. IR spectra were recorded (KBr pellet) on Shimadzu Prestige 21 FTIR instrument in the range of 4000 to 400 $cm^{-1}$. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded using a Bruker-Avance 300 MHz FT-NMR spectrometer (300 and 75 MHz, respectively) using DMSO-$d_6$ as solvent and TMS as internal standard. Chemical shifts (δ) were mentioned in parts per million (ppm). The following abbreviations are used: s-singlet, d-doublet, t-triplet, q-quintet and m-multiplet. Low resolution mass spectra were recorded on Agilent 6110LC/MS mass spectrophotometer using ESI mode. The elemental analysis was done (sample thoroughly dried under vacuum) using a Thermo Fischer Flash 1112 Series elemental analyzer. For antimicrobial activity, the reference cultures were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India-160 036 and the remaining cultures were prevailed from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

**Experimental procedure for the synthesis of compound (3):** Thionyl chloride (8 mL, 0.11 mol) was added to bosentan **2** (55g, 0.10 mol) in chloroform (500 mL) and heated to 55 to 60 °C for 3 h under nitrogen. Excess thionyl chloride and chloroform were completely knocked off under vacuum to get a yellow solid which was filtered and washed with acetone (120 mL). Obtained 48g of solid after drying.

**4-tert-Butyl-N-(6-(2-chloroethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)benzene- sulfonamide (3):** Yellow solid. Yield 85%. Mp: 198-200 $^{o}C$; IR: 3252, 2954, 2904, 2835, 1684, 1618, 1570, 1564, 1554, 1499, 1455, 1438, 1397, 1384, 1284, 1253, 1213, 1173, 1162, 1155, 1111, 1083, 1026, 993, 874, 833, 827, 741, 698, 679, 625, 572, and 545 $cm^{-1}$. $^{1}H$-NMR (400 MHz, DMSO-$d_6$): $\delta_H$: 1.36 (s, 9H), 3.88 (s, 3H), 4.51 (t, 2H, *J*=8.0 Hz), 5.18 (t, 2H, *J*=8.0 Hz), 6.76–10.02 (m, 11H, aromatic) and 11.62 (bs, 1H). $^{13}C$-NMR (100 MHz, DMSO-$d_6$): $\delta_C$: 31.33, 35.41, 37.73, 52.59, 56.27, 113.48, 115.93, 120.76, 123.86, 124.58, 126.04, 127.33, 129.15, 138.24, 141.61, 145.71, 147.01, 149.26, 149.52, 153.73, 155.09, 156.59 and 165.98. ESI-MS:

$[M+H]^+$ at *m/z* 570 ($Cl^{35}$) & 572 ($Cl^{37}$) in 3:1 ratio. Anal. Calcd for $C_{27}H_{28}ClN_5O_5S$: C, 56.89; H, 4.95; N, 12.29. Found C, 57.12; H, 4.99; N, 12.35.

**Experimental procedure for the synthesis of azide (4):**
N,N-dimethylacetamide (250 ml) was charged to **3 (**46g, 0.08 mol), followed by sodium azide (6.5g, 0.1 mol) and stirred at ambient temperature for 16 to 20 h. Completion of the reaction was confirmed by TLC (10% methanol in ethyl acetate) after which, 500 mL of water was added to the reaction mass and the obtained solid was filtered and dried under vacuum. Obtained azide **4** (43g) was preserved in a refrigerator.

**N-(6-(2-Azidoethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)-4-tert-butylbenzenesulfonamide – [azide 4]:** Pale brown solid. Yield 93%. Mp: 172-175 °C. IR: 3245, 3066, 2959, 2903, 2835, 2795, 2090, 1681, 1617, 1563, 1550, 1499, 1455, 1397, 1386, 1333, 1254, 1215, 1179, 1162, 1111, 1084, 926, 873, 833, 824, 768, 749, 625, 574 and 545 $cm^{-1}$. $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$: 1.26 (s, 9H), 3.47 (t, 2H, *J*=6.2 Hz), 3.83 (s, 3H), 4.12 (t, 2H, *J*=18.3 Hz), 6.67–9.11 (m, 11H, aromatic), 11.30 (bs, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta_C$: 31.23, 35.24, 44.34, 49.06, 56.18, 113.35, 115.33, 120.85, 122.84, 123.29, 124.33, 125.60, 128.53, 138.48, 146.14, 146.92, 149.33, 151.31, 156.26, 157.27, 158.38 and 159.58.ESI-MS: $[M+H]^+$ at *m/z* 577. Anal. Calcd for $C_{27}H_{28}N_8O_5S$: C, 56.24; H, 4.89; N, 19.43. Found C, 56.55; H, 4.93; N, 19.52.

**General experimental procedure for one pot synthesis of (1a-f):** Amine **5a-f** (0.010 mol), n-butanol (10 mL), potassium carbonate (1.7g, 0.012 mol, pre-dried at 100 °C) and propargyl chloride (0.75g, 0.010 mol) were mixed and heated at 55 to 60 °C for 6 h. Cooled the mass to 20 °C and charged with water (10 mL), DIPEA (1.3g, 0.02 mol), copper iodide (200mg, 10 mol%) followed by azide **4** (5.8g, 0.01 mol) and agitated at ambient temperature for 6 to 10 h. Completion of the reaction was checked by TLC (10% methanol/ethyl acetate). To the mass, 50 mL of water and 50 mL of ethyl acetate were charged, stirred and filtered to remove inorganic. The organic layer was separated, dried over anhydrous sodium sulphate followed by vacuum distillation using a Rota evaporator at below 60 °C, yielded a semi solid. The title compounds **1a-f** were obtained as pale brown to brown amorphous solid in between 78-91% yields, when it was triturated with n-heptane, filtered and dried under vacuum at 60 °C.

**4-tert-Butyl-N-(6-(2-(4-((((1S,2R)-1-hydroxy-1-phenyl-2-propyl)(methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)-ethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)-benzenesulfonamide (1a**): Pale brown amorphous solid. Yield 6.7g (88%). Mp: 122-125 °C. IR: 3373, 2961, 2924, 2853, 1658, 1651, 1587, 1562, 1520, 1499, 1454, 1395, 1250, 1217, 1177, 1138, 1103, 1078, 851, 750, 702, 629 and 577 $cm^{-1}$. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 0.90 (d, 3H, *J=14.4 Hz*), 1.01 (s, 9H), 2.71 (t, 2H, *J*=12.2 Hz), 3.29 (s, 2H), 3.81 (s, 3H), 4.19 (s, 3H), 4.64 (t, 2H), 4.98 (m, 1H), 5.79 (bs, 1H), 6.78-7.72 (m, 17H, aromatic) and 8.97 (bs, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 21.59, 26.92, 27.17, 29.75, 30.03, 31.59, 34.73, 48.15, 56.15, 63.48, 113.18, 113.29, 113.55, 113.77, 120.84, 120.86, 124.41, 124.55, 125.18, 125.25, 126.29, 126.32, 126.69, 126.76, 126.79, 127.03, 127.10, 127.89, 128.15, 128.67 and 157.98. ESI-MS *m/z* 780 $[M+H]^+$. Anal. Calcd for $C_{40}H_{45}N_9O_6S$: C, 61.60; H, 5.82; N, 16.16.. Found C, 62.20; H, 5.87; N, 16.10.

**4-tert-Butyl-N-(6-(2-(4-(((1S,2R)-1-hydroxy-1-phenyl-2-propyl-amino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)-5-(2-methoxy-phenoxy)-2,2'-bipyrimidin-4-yl)benzenesulfonamide(1b):** Brown amorphous solid. Yield 6.0g (78%). Mp: 140-143 °C. IR: 3356, 2959, 2924, 2853, 1668, 1603, 1562, 1499, 1456, 1393, 1341, 1252, 1219, 1175, 1140, 1103, 1080, 1022, 908, 852, 750, 702, 627, 575 and 548 $cm^{-1}$. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$0.94 (d, 3H, *J=10.5 Hz*), 1.24 (s, 9H), 3.18 (s, 2H), 3.83 (s, 3H), 4.11 (t, 2H, *J*=12.2 Hz), 4.59 (t, 2H, *J*=12.2 Hz), 5.10 (d of d, 1H, *J*=56 Hz & *J*=20.2 Hz), 5.92 (m, 1H), 6.56–7.59 (m, 17H), 8.94 (bs, 1H) and 9.00 (bs, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 14.33, 22.62, 29.46, 29.90, 31.40, 31.76, 32.02, 34.86, 56.14, 113.23, 114.51, 115.11, 116.05, 120.35, 120.84, 123.19, 123.64, 125.15, 126.31, 127.84, 128.19, 128.21, 128.24, 128.32, 128.66, 128.99, 129.92, 131.86, 133.48, 139.58, 153.83 and 158.22. ESI-MS *m/z* 766 $[M+H]^+$. Anal. Calcd for $C_{39}H_{43}N_9O_6S$: C, 61.16; H, 5.66; N, 16.46. Found C, 61.30; H, 5.69; N, 16.50.

**4-tert-Butyl-N-(5-(2-methoxyphenoxy)-6-(2-(4-((methyl(1-phenylpropan-2-yl)-amino)methyl)-1H-1,2,3-triazol-1-yl)-ethoxy)-2,2'-bipyrimidin-4-yl)benzenesulfonamide(1c):** Brown amorphous solid. Yield 6.9g (90%). Mp: 99-102 °C. IR: 3447, 3063, 2961, 2924, 2853, 1653, 1595, 1562, 1499, 1458, 1393, 1250, 1221, 1175, 1140, 1107, 1076, 849, 746, 702, 631, 577 and 546 $cm^{-1}$. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$1.04 (s, 3H), 1.24 (s, 9H), 2.62 (t, 2H, *J*=11.3 Hz), 3.14 (t, 2H, *J*=15.3 Hz), 3.48 (s, 3H), 3.83 (s, 3H), 4.18 (1H, m), 4.24 (d of d, 2H), 4.65 (t, 2H), 6.60–7.73 (m, 17H, aromatic) and 9.01 (bs, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 29.47, 29.90, 30.34, 31.39, 31.76, 32.09, 34.99, 36.07, 45.11, 56.11, 113.13, 114.37, 120.84, 122.46, 122.54, 122.80, 124.79, 124.95, 126.92, 127.50, 127.78, 128.08, 128.17, 128.91, 129.08, 129.72, 130.12, 133.33, 153.45 and 158.15. ESI-MS *m/z* 764 $[M+H]^+$. Anal. Calcd for $C_{40}H_{45}N_9O_5S$: C, 62.89; H, 5.94; N, 16.50. Found C, 62.85; H, 5.98; N, 16.55.

**4-tert-Butyl-N-(6-(2-(4-(((2-hydroxy-2-(3-hydroxyphenyl)-ethyl)(methyl)amino)-methyl)-1H-1,2,3-triazol-1-yl)ethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)-benzenesulfon-amide (1d):** Brown amorphous solid. Yield 7.1g (90%). Mp 148-150 °C. IR: 3443, 3067, 2963, 2868, 1653, 1693, 1593, 1564, 1499, 1456, 1396, 1252, 1217, 1180, 1138, 1103, 1078, 1047, 906, 837, 752, 702, 630 and 577 $cm^{-1}$. ESI-MS *m/z* 782 $[M+H]^+$. Anal. Calcd for $C_{39}H_{43}N_9O_7S$: C, 59.91; H, 5.54; N, 16.12. Found C, 60.05; H, 5.57; N, 16.24.

**4-tert-butyl-N-(6-(2-(4-((2,3-dihydro-1H-inden-1-ylamino)-methyl)-1H-1,2,3-triazol-1-yl)ethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)benzenesulfonamide(1e):** Yellowish brown solid. Yield 6.6g (88%). Mp: 102-105 °C. IR: 3422, 2963, 2868, 1674, 1605, 1564, 1499, 1458, 1393, 1340, 1252, 1219, 1173, 1111, 1084, 1020, 864, 837, 762, 625 and 575 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 1.25 (s, 9H), 2.24 &2.41 (m, 2H, *J*=7.5 Hz), 2.87 (m, 2H), 3.14 (m, 2H), 3.82 (s, 3H), 4.22 (s, 2H), 4.25 (t, 2H, *J*=8.0 Hz), 4.73 (t, 2H, *J*=12 Hz), 6.67–8.24 (m, 16H, aromatic) and 9.75 (bs, 2H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 28.80, 28.90, 30.28, 31.28, 35.21, 36.61, 45.35, 56.14, 61.13, 97.74, 104.52, 108.11, 113.21, 115.20, 120.80, 125.45, 125.50, 125.53, 125.57, 126.47, 126.71, 127.05, 127.16, 128.45, 129.91, 137.73, 138.20, 138.79, 145.41, 149.17, 157.42, 158.36 and 159.24. ESI-MS *m/z* 748 $[M+H]^+$. Anal. Calcd for $C_{39}H_{41}N_9O_5S$: C, 62.63; H, 5.53; N, 16.86. Found C, 62.53; H, 5.59; N, 16.94.

**N-(1-(2-(1-((6-(4-tert-Butylphenylsulfonamido)-5-(2-methoxy-phenoxy)-2,2'-bipyrimidin-4-yl)methyl)-1H-1,2,3-triazol-4-yl-oxy)ethyl)-4-(methoxymethyl)piperidin-4-yl)-N-phenylpropio-namide (1f):**Pale brown solid. Yield 8.1g (91%). Mp 180-181 °C. IR: 3474, 2965, 2833, 1651, 1589, 1562, 1518, 1499, 1456, 1396, 1377, 1323, 1250, 1229, 1178, 1140, 1107, 1078, 1002, 849, 750, 706, 633, 579 and 552 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 0.79 (t 3H, *J*=7.3 Hz), 1.23 (s, 9H), 1.69,(t, 4H), 1.98 (t, 4H), 3.32 (s, 3H), 3.52 (q, 2H), 3.85 (t, 2H), 3.93 (s, 2H), 4.12 (s, 2H), 4.56 (t, 2H), 6.51-7.74 (m, 17H) and 8.95 (bs, 1H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta_C$9.97, 23.30, 28.29, 28.70, 30.47, 31.53, 31.59, 34.80, 59.28, 76.57, 79.46, 89.08, 113.80, 120.86, 122.40, 124.47, 129.13, 131.52, 133.47, 134.01, 135.10, 136.29, 139.18, 140.51, 141.35, 146.09, 149.15, 150.32, 152.92 and 168.34. ESI-MS *m/z* 891 $[M+H]^+$.Anal. Calcd for C46H54N10O7S: C, 62.00; H, 6.11; N, 15.72. Found C, 62.39; H, 6.15; N, 15.89.

**General experimental procedure for one pot synthesis of 1g-k**
In a nitrogen atmosphere, thionyl chloride (0.8 mL, 0.011 mol) was added to acid **5g-k** (0.010 mol) in chloroform (10 mL) at 50 to 55 °C for 3 to 4 h. Then it was cooled to about 10 °C and propargyl alcohol (0.56g, 0.010 mol) was added and stirred for 4-6 h at ambient temperature. Further it was heated to 50 °C and the solvent was distilled off completely under vacuum and the obtained residue was charged with n-butanol (10 mL), water (10 mL), DIPEA (2.6g, 0.02 mol) and copper iodide (200mg, 10 mol%) followed by azide **4** (5.8g, 0.01 mol) and stirred at ambient temperature for 6 to 10 h. Completion of the reaction was checked by TLC (10% methanol/ethyl acetate). To the mass, 50 mL of water and 50 mL of ethyl acetate were charged, stirred and filtered to remove inorganic. Organic layer was separated, dried over anhydrous sodium sulphate followed by vacuum distillation using a rota evaporator at below 60 °C yielded a semi solid. The title compounds **1g-k** were obtained as a pale brown to brownish amorphous solids in between 84-92% yield when it was triturated with n-heptane, filtered and dried under vacuum at 60 °C.

**(1-(2-(6-(4-tert-Butylphenylsulfonamido)-5-(2-methoxyphen-noxy)-2,2'-bipyrimidin-4-yl-oxy)ethyl)-1H-1,2,3-triazol-4-yl)-methyl benzoate (1g**): Yellowish brown solid. Yield 6.8g (92%). Mp: 95-98 °C. IR: 3144, 3067, 2963, 2926, 2868, 2853, 1719, 1676, 1605, 1564, 1499, 1452, 1393, 1340, 1271, 1254, 1219, 1173, 1111, 1084, 1024, 864, 835, 752, 714, 692, 625, 573 and 552 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 1.25 (s, 9H), 3.84 (s, 3H), 4.34 (t, 2H, *J*=9.5 Hz), 4.66 (t, 2H, *J*=8.0 Hz), 5.32 (s, 2H), 6.70-9.03 (m, 17H, aromatic) and 11.30 (bs, 1H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 31.23, 35.25, 51.34, 56.16, 58.33, 59.58, 113.24 to 165.90. ESI-MS *m/z* 737 $[M+H]^+$. Anal. Calcd for $C_{37}H_{36}N_8O_7S$: C, 60.31; H, 4.92; N, 15.21. Found C, 60.25; H, 4.96; N, 15.20.

**(1-(2-(6-(4-tert-Butylphenylsulfonamido)-5-(2-methoxyphe-noxy)-2,2'-bipyrimidin-4-yl-oxy)ethyl)-1H-1,2,3-triazol-4-yl)-methyl 2-bromo-5-methoxybenzoate (1h):** Pale brown solid. Yield 7.2g (84%). Mp: 160-164 °C. IR: 3068, 2965, 2905, 1709, 1674, 1602, 1565, 1515, 1500, 1464, 1456, 1419, 1394, 1343, 1290, 1271, 1253, 1220, 1176, 1140, 1111, 1084, 1022, 869, 835, 763, 753, 696, 627, 575 and 547 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 1.26 (s, 9H), 3.77 (s, 3H), 3.83 (s, 3H), 4.32 (t, 2H), 4.67 (t, 2H), 5.32 (s, 2H), 6.69-9.03 (m, 15H, aromatic) and 11.30 (bs, 1H).$^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 31.24, 35.24, 45.69, 45.76, 47.91, 56.20, 58.98, 110.74, 113.29, 115.52, 116.56, 119.56, 120.85, 122.75, 123.23, 124.44, 125.65, 125.91, 128.52, 133.26, 135.26, 138.38, 146.09, 146.69, 149.27, 151.17, 156.31, 157.38, 158.26, 158.85, 159.10 and 165.60. ESI-MS *m/z* 847 $[M+H]^+$. Anal. Calcd for $C_{38}H_{37}BrN_8O_8S$; C, 59.60; H, 4.87; N, 14.63. Found C, 59.69; H, 4.93; N, 14.70.

**(1-(2-(6-(4-tert-Butylphenylsulfonamido)-5-(2-methoxyphe-noxy)-2,2'-bipyrimidin-4-yl-oxyethyl)-1H-1,2,3-triazol-4-yl)-methyl-4-nitrobenzoate (1i):** Brown solid. Yield 7.9g (88%). Mp: 168-171 °C. IR: 3111, 3078, 2965, 2869, 2838, 1726, 1675, 1607, 1565, 1528, 1499, 1456, 1393, 1342, 1267, 1253, 1219, 1172, 1112, 1102, 1084, 1014, 872, 858, 762, 752, 720, 625, 575 and 546 $cm^{-1}$.$^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 1.25 (s, 9H), 3.83 (s, 3H), 4.34 (t, 2H, *J*=9.5 Hz), 4.67 (t, 2H, *J*=8.0 Hz), 5.38 (s, 2H), 6.69-9.03 (m, 16H, aromatic) and 11.31 (bs, 1H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 31.58, 35.24, 47.82, 56.13, 59.05, 59.26, 124.39, 124.75, 125.46, 125.78, 127.62, 128.51, 131.22, 131.86, 132.12, 135.23, 142.50, 142.81, 146.92, 148.26, 150.77, 158.25, 160.23, 161.22, 161.58, 162.12, 164.45 and 164.56; ESI-MS *m/z* 891 $[M+H]^+$. Anal. Calcd for C37H35N9O9S: C, 56.84; H, 4.51; N, 16.12. Found C, 56.98; H, 4.55; N, 16.19.

**(1-(2-(6-(4-tert-Butylphenylsulfonamido)-5-(2-methoxyphen-oxy)-2,2'-bipyrimidin-4-yl-oxyethyl)-1H-1,2,3-triazol-4-yl)-methyl 3,4-dimethoxybenzoate (1j):** Brown solid. Mp 128-132 °C. Yield 7.2g (90%). IR: 3605, 3150, 3076, 2964, 2905, 2837, 1707, 1675, 1601, 1565, 1514, 1500, 1457, 1418, 1392, 1343, 1289, 1271, 1253, 1219, 1174, 1111, 1022, 868, 834, 763, 694, 626, 575 and 546 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta_H$1.26 (s, 9H), 3.80 (s, 6H), 4.32 (t, 2H), 4.66 (t, 2H), 5.30 (s, 2H), 6.71-9.02 (m, 17H, aromatic) and 11.30 (bs, 1H). ESI-MS: $[M+H]^+$ at

*m/z* 797; Anal. Calcd for $C_{39}H_{40}N_8O_9S$: C, 58.78; H, 5.06; N, 14.06. Found C, 58.69; H, 5.12; N, 14.04.

**(1-(2-(6-(4-tert-Butylphenylsulfonamido)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl-oxy)ethyl)-1H-1,2,3-triazol-4-yl)-methyl 3-methylbenzoate (1k):** Brown solid. Mp 140-144 $^{o}$C.Yield 6.6g (87%). IR: 3455, 3139, 3067, 2964, 2904, 2869, 1718, 1653, 1562, 1500, 1457, 1397, 1278, 1251, 1228, 1214, 1202, 1179, 1141, 1105, 1081, 1021, 851, 835, 748, 668, 633, 580 and 551 cm$^{-1}$. $^{1}$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 1.23 (s, 9H), 2.36 (s, 3H), 3.83 (s, 3H), 4.16 (t, 2H), 4.62 (t, 2H), 5.32 (s, 2H), 6.57-8.07 (m, 15H, aromatic) and 8.95 (bs, 1H). ESI-MS: $[M+H]^+$ at *m/z* 751; Anal. Calcd for $C_{38}H_{38}N_8O_7S$: C, 60.79; H, 5.10; N, 14.92. Found C, 60.95; H, 5.14; N, 14.96.

**General experimental procedure for the synthesis of 1l-n:** Aliphatic terminal alkyne **5l-n** (0.01 mol), n-butanol (6 mL), water (6 mL), DIPEA (2.6g, 0.02 mol) and copper iodide (200mg, 10 mol%) were mixed followed by azide **4** (5.8g, 0.01 mol) and agitated at ambient temperature for 6 to 10 h. Completion of the reaction was checked by TLC (10% methanol in ethyl acetate). To the mass, 50 mL of water and 50 mL of ethyl acetate were charged, stirred and filtered to remove inorganics. Organic layer was separated, dried over anhydrous sodium sulphate followed by vacuum distillation using a rota evaparotor at below 60 °C yielded a semi solid. The title compounds, **1l-n** were obtained as a pale brown to brown amorphous solids in between 87-95% yield when the semi solid was triturated with n-heptane, filtered and dried under vacuum at 60 °C.

**4-tert-Butyl-N-(6-(2-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)-ethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)-benzenesulfonamide (1l):** Yellowish brown solid. Mp 168-170 $^{o}$C. Yield 6.0g (95%).IR: 3447, 3134, 2965, 2870, 2835, 1678, 1661, 1609, 1568, 1499, 1427, 1394, 1348, 1250, 1219, 1196, 1167, 1113, 1084, 1026, 856, 833, 756, 692, 625, 573 and 546 cm$^{-1}$.$^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 1.27 (s, 9H), 3.84 (s, 3H), 4.30 (t, 2H, *J*=9.5 Hz), 4.45 (t, 2H, *J*=9.5 Hz), 4.61 (s, 2H), 5.13 (bs, 1H), 6.70-9.05 (m, 12H, aromatic) and 11.27 (bs, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 22.41, 28.69, 47.77, 55.46, 69.75, 71.11, 111.40, 113.65, 115.24, 118.88, 123.45, 126.56, 128.54, 128.68, 129.04, 134.68, 136.43, 143.13, 143.91, 147.00, 148.41, 150.75, 157.66 and 161.20.ESI-MS: $[M+H]^+$ at *m/z* 633; Anal. Calcd for $C_{30}H_{32}N_8O_6S$: C, 56.95; H, 5.10; N, 17.71. Found C, 57.15; H, 5.14; N, 17.85.

**4-tert-Butyl-N-(6-(2-(4-butyl-1H-1,2,3-triazol-1-yl)ethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)benzenesulfonamide (1m):** Brown solid. Mp 101-103 °C.Yield 5.9g (89%). IR: 3136, 3069, 2959, 2870, 1676, 1605, 1564, 1499, 1456, 1393, 1340, 1252, 1219, 1173, 1111, 1084, 1045, 1022, 864, 837, 752, 694, 625, 575 and 546 cm$^{-1}$.$^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 0.88 (t, 3H, *J*=6.8 Hz), 1.27 (m, 13H, aromatic), 1.51 (t, 2H, *J*=7.5 Hz), 3.83 (s, 3H), 4.31 (t, 2H, *J*=9.5 Hz), 4.57 (t, 2H, *J*=9.5 Hz), 6.71-9.04 (m, 12H, aromaitc) and 11.27 (bs, 1H). ESI-MS: $[M+H]^+$ at *m/z* 659. Anal. Calcd for $C_{33}H_{38}N_8O_5S$: C, 60.17; H, 5.81; N, 17.01. Found C, 60.85; H, 5.86; N, 17.15.

**4-tert-Butyl-N-(6-(2-(4-(1-hydroxycyclohexyl)-1H-1,2,3-triazol-1-yl)ethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl)-benzenesulfonamide (1n):** Brown solid. Mp 105-107 $^{o}$C. Yield 5.9g (87%). IR: 3418, 3140, 3069, 2936, 2860, 1674, 1607, 1566, 1499, 1456, 1393, 1340, 1285, 1252, 1219, 1171, 1111, 1084, 1047, 1020, 866, 835, 752, 702, 625, 575 & 546 cm$^{-1}$. $^{1}$H-NMR (400 MHz, DMSO-$d_6$): $\delta_H$ 1.27 (m, 11H), 1.41 (m, 2H), 1.64 (m, 4H, *J*=5.2 Hz), 1.78 (m, 2H), 3.85 (s, 3H), 4.29 (t, 2H, *J*=5.6 Hz), 4.60 (m, 2H, *J*=6.4 Hz), 4.77 (bs, 1H), 6.74-9.06 (m, 12H, aromatic), 11.27 (bs, 1H); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 22.06, 25.72, 31.24, 35.24, 38.19, 45.89, 47.42, 56.21, 68.41, 113.30, 115.49, 120.90, 121.89, 122.84, 123.22, 124.42, 125.62, 128.51, 138.43, 146.15, 146.80, 147.52, 149.29, 151.25, 156.25, 157.41, 158.32 & 159.15;ESI-MS: $[M+H]^+$ at *m/z* 683; Anal. Calcd. for $C_{35}H_{40}N_8O_6S$: C, 59.98; H, 5.75; N, 15.99.Found C, 60.12; H, 5.79; N, 16.11.

**Biological assays**

**Materials and methods for antimicrobial activity:** Streptomycin and Ciprofloxacin (Sigma) were used as positive control against bacteria. Ketoconazole (Himedia, Mumbai) was used as a positive control against fungi.

**Microbes**: The following bacteria and fungi were used for the experiment. Bacteria: *Bacillus subtilis* MTCC 44.1, *Klebsiella pneumoniae* MTCC 109, *Staphylococcus epidermidis* MTCC 3615, *Micrococcus luteus* MTCC 106, *Salmonella typhimurium* MTCC 1251, *Proteus vulgaris* MTCC 1771, *Shigellaflexneri* MTCC 1457, *Enterobacter aerogenes* MTCC 111, *Staphylococcus aureus* MTCC 96, *Pseudomonas aeruginosa* MTCC 741 and *Staphylococcus aureus* (MRSA-*methicillin resistant*). The reference cultures were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh-160036, India; Fungi: *Candida albicans* MTCC 227 and *Malassesia-pachydermatis.* All the remaining cultures were prevailed from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India.

***In vitro* well method**

Petri plates were prepared with 20 mL of sterile Mueller Hinton agar (MHA) (Hi-media, Mumbai). The test cultures were swabbed on top of a solidified media and were allowed to dry for 10 min and a specific amount of synthesised compound at 1mg/disc was added to each well separately. Negative control was prepared using respective solvents. Streptomycin was used as positive control against bacteria. Ketoconazole was used as positive control for fungi. The plates were incubated for 24 h at 37°C for bacteria and for 48 h at 28°C for fungi. Zones of inhibition were recorded in mm and the experiment was repeated twice. Bacterial inoculums were prepared by growing cells in Mueller Hinton broth (MHB) (Himedia) for 24 h at 37°C. The filamentous fungi were grown on Sabouraud dextrose agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and were

homogenized. Yeast was grown on a Sabouraud dextrose broth (SDB) at 28°C for 48 h.

**Minimum inhibition concentration study:**

The required concentrations (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL, 15.62 μg/mL and 7.81 μg/mL) of the compound were dissolved in DMSO (2%), and were diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100 μL from each well was inoculated. The antifungal agents, Ketoconazole for fungi and Streptomycin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48 to 72 h at 28 °C and for bacteria the plates were incubated for 24 h at 37 °C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 μL of the tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

**Study of cytotoxicity**

A549 (Lung), SKOV-3 (Ovarian) and Vero cells were obtained from ATCC, USA. A549, SKOV-3 and Vero cells were maintained in complete tissue culture medium DMEM with 10 % Fetal Bovine Serum and 2mM L-Glutamine, along with antibiotics (about 100 IU/mL of penicillin, 100 μg/mL of streptomycin) with the pH adjusted to 7.2. The cytotoxicity was determined according to the method[57] with some changes. Cells ($5\times10^5$) were seeded in 96 well plates containing medium with different concentrations such as 250, 125, 62.5, 31.25, 15.63 and 7.81 μg/mL. The cells were cultivated at 37°C with 5% $CO_2$ and 95% air in 100% relative humidity. After various durations of cultivation, the solution in the medium was removed. An aliquot of 100 μL of medium containing 1 mg/mL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) was loaded to the plate. The cells were cultured for 4 h and then the solution in the medium was removed. An aliquot of 100 μL of DMSO was added to the plate, which was shaken until the crystals were dissolved. The cytotoxicity against cancer cells was determined by measuring the absorbance of the converted dye at 570 nm in an ELISA reader. Cytotoxicity of each sample was expressed as $IC_{50}$ value. The $IC_{50}$ value is the concentration of test sample that causes 50% inhibition of cell growth, averaged from three replicate experiments. The percentage of growth inhibition was calculated using the following formula; Inhibition (%) = A-B /A x 100 (A-Control group and B-Treated group).

**Molecular docking study**

Molecular docking studies have been done using the AutoDock Tools (ADT) version 1.5.6 and AutoDock version 4.2.5.1 docking program. The DNA topoisomerase IV and ALK structures were obtained from the Protein Data Bank (PDB ID: 4EMV and 2XP2). The co-crystallized ligands in the receptors were removed. Then, the polar hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT. Further ADT was used to remove crystal water, Gasteiger charges were added to each atom, and merged the non-polar hydrogen atoms to the protein structures. The distance between the donor and an acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor–hydrogen–donor angle was not less than 120˚. The structures were then saved in PDBQT file format, for further studies in ADT. The grid boxes with dimension of 40 × 40 × 40 $Å^3$and 60 × 60 × 60 $Å^3$with 0.375 Å spacing and centred on 14.860, 29.555, 6.941 and 29.697, 47.794, 8.863was created around the binding site of co-crystallised ligand on 4EMV and 2XP2 respectively. The centre of the box was set at co-crystallised ligand centre and grid energy calculations were carried out.In order to verify the reproducibility of the docking calculations, the bound ligand was extracted from the complexes and submitted for one-ligand run calculation. This reproduced top scoring conformation falling within root-mean-square deviation (RMSD) value of 0.58 to 1.53 Å and 1.63 to 1.95 Å with bound X-ray conformation for 4EMV and 2XP2 respectively, suggesting this method is valid enough to be used for docking studies of other compounds. Docking of different ligands to protein was performed using AutoDock, following the same protocol used in as that of validation study. All dockings taken into 2.5 million energy evaluations were performed for each of the test molecules. For each compound, 50 docked conformations were generated. The energy calculations were done using genetic algorithms. Docked ligand conformations were analyzed in terms of energy, hydrogen bonding, and hydrophobic interaction between ligand and receptor. Detailed analyses of the ligand-receptor interactions were carried out, and final coordinates of the ligand and receptor were saved. For display of the receptor with the ligand binding site, PyMOL software[63] was used. From the docking scores, the free energy of binding (FEB) of all compounds were calculated.

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# Synthesis of Novel 1,4-Disubstituted 1,2,3-Triazolo-Bosentan Derivatives – Evaluation of Antimicrobial, Anticancer activities and Molecular Docking

K. Easwaramoorthi,[a] A. Jeya Rajendran,*[,a] K. ChennakesavaRao,[b] Y. Arun,[b] C. Balachandran,[c] P. T. Perumal,[b] Nobuhiko Emi,[c] S.M. Mahalingam,[d] V.Duraipandiyan,[e,f] N.A. Al-Dhabi[f]

[a]*Department of Chemistry, Loyola College, Chennai-600034, India, Phone: +91 944-411-6528, Email: jeyaadmaterial@gmail.com*

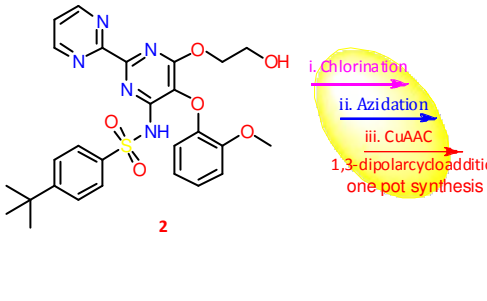
[b]*Organic Chemistry Division, CSIR-CLRI, Chennai-600020, India*

[c]*Department of Hematology, Fujita Health University, 1-98, Dengakugakubo, Kutsukake-cho, Toyoake,Aichi 470-1192, Japan.*

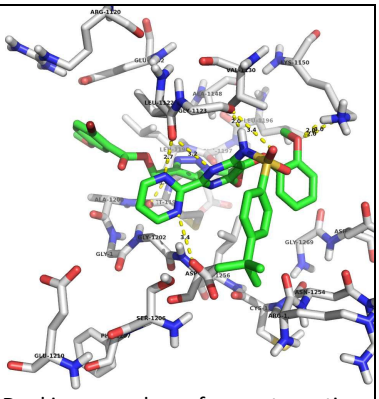
[d]*Centre for Drug Discovery and Department of Chemistry, Purdue University, Indiana*

[ef]*Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai-600034, India*

[f]*Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saud University,P.O.Box.2455, Riyadh-11451, Saudi Arabia.*




*One pot synthesis with good yeids
*Good antimicrobial activity against 4EMV receptot
*Prominet anticancer activity against A549 and SKOV-3 cellines
*Significant *in vitro* cytotoxicity at 7.81µg/mL



Docking mode of most active compound **1h** with 2XP2 recptor