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Anti Fungal and Acute Toxicity Activities of Novel 2,4,6-Trisubstituted Pyrimidines.

[1*]Reddy Rambabu, [2]Y Rajendra Prasad, [1]S Vidyadhara.

[1*]Department of Pharmaceutical Chemistry, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India, [2]Department of Pharmaceutical Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Abstract

Frequency of microbial infection is progressively increasing worldwide. New emerging strain of bacterium and resistance to currently available drugs make this field more conscientious and alarming. Effective antimicrobials are available for treatment since long back. As toxicity became a major criteria for usage of drugs now a days, many potent antibiotic are of restricted use in clinical practice. Pyrimidine is an aromatic heterocyclic organic compound similar to pyridine. A new class of heterocyclic 2, 4, 6 tri substituted pyrimidines were prepared from chalcones. The Structures of these compounds were established on the basis of IR and ^{1}H NMR data. Synthesized compounds were evaluated for antimicrobial and cytotoxicity activities. Among all, 2",4"- di fluoro phenyl substituted pyrimidine derivative have shown significant antifungal activity against pathogenic fungi like *Aspergillus niger*, *Candida tropicalis* and most compounds have shown no significant cytotoxicity in HT-29, MCF-7 and DU-145 cell lines. This impressive cytotoxicity and anti microbial action encourages us to synthesize novel pyrimidines for desired action.

Key Words

2,4,6-trisubstituted pyrimidine, Chalcone, *Aspergillus niger* and *Candida tropicalis.*

Introduction

The pyrimidine ring system has wide occurrence in nature as substituted and ring fused compounds and derivatives, including the nucleotides, thiamine (vitaminB1) and alloxan[1]. It is also found in many synthetic compounds such as barbiturates and the HIV drug, zidovudine. Although pyrimidine derivatives such as uric acid and alloxan were known in the early 19th century, a laboratory synthesis of a pyrimidine was not carried out until 1879, when Grimaux reported the preparation of barbituric acid from urea and malonic acid in the presence of phosphorus oxychloride. The systematic study of pyrimidines began in 1884 with Pinner, who synthesized derivatives by condensing ethyl acetoacetate with amidines. Pinner first proposed the name "pyrimidin" in 1885[2-6]. The parent compound was first prepared by

***Corresponding Author:**
reddy.rambabu1155@gmail.com

Gabriel and Colman in 1900, by conversion of barbituric acid to 2,4,6-trichloropyrimidine followed by reduction using zinc dust in hot water. The finding that 2,4-diaminopyrimidines inhibit the growth of microorganisms by interfering with their utilization of folic acid led to an intensive search for antiinfective agents in this class of heterocyclic compounds. Trimethoprim developed as an antimalarial drug had unique broad spectrum antimicrobial action. The pioneering work of Hitchings led to the combination of trimethoprim with sulfa drug, sulfamethoxazole constituting an important advance in the development of clinically effective antimicrobial agents. Chemical modification of trimethoprim led to potent antibacterial compound tetroxoprim[6-10]. They synthesized some 2-tosylamino and 2-tosyliminopyrimidine derivatives and studied their interference with some leukocyte functions and 5-lipooxygenase (5-LOX) activity. The study demonstrated that all the compounds inhibited cell free 5-LOX activity and reduced activation of neutrophils, which may have relevance for the modulation of the inflammatory response[11-14].

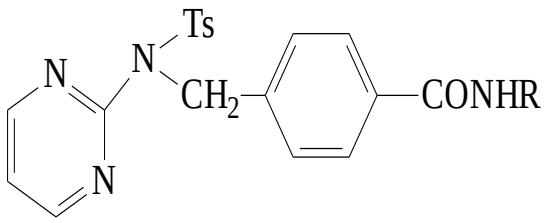
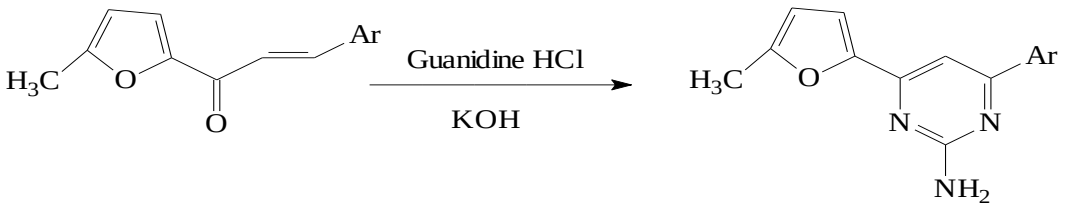


Fig. 1: Pyrimidine derivative.

Materials and Methods

General procedure for the synthesis of pyrimidines[15-18]

The condensation of the chalcones with guanidine hydrochloride in an alkaline medium viz., in potassium hydroxide in the presence of ethanol, at reflux temperatures (2 to 6 hr) resulted in the formation of corresponding pyrimidines. Completion of the reaction was established by TLC using silica gel-G. After completion of the reaction, the reaction mixture was poured onto crushed ice with constant stirring. The solid that separated was filtered, dried and purified by column chromatography on silica gel, using a mixture of ethyl acetate and hexane as the mobile phase[19-21]. The purified pyrimidine derivatives were obtained as light to bright yellow fine powders was show in Fig. 2.



Chalcone **Trisubstituted pyrimidines**

Figure 2: Synthesis of Tri substituted pyrimidine.

Antimicrobial Activities

Antifungal activity

Potato dextrose agar (Hi-media) was dissolved and distributed in 25 ml quantities in 100ml conical flasks and were sterilized in an autoclave at 121^0C (15lbs/sq.in) for 20 minutes. The medium was inoculated at one percent level using 48hrs old cultures of the test organism mentioned above aseptically into sterile petridish and allowed to set at room temperature for about 30 minutes. In a size of 4 inches petridish, cups of 8mm diameter at equal distance were made in each plate. In each plate, one cup was used for control i.e. Dimethyl sulfoxide (DMSO), other for standard Fluconazole with 100µg/ml and remaining with concentrations of test compound i.e. 4,16,64,128, 256 and 512 µgm/ml solutions. The plates thus prepared were left for 90 minutes in refrigerator for diffusion .After incubation for 48 hours at 25^0C, the plates were examined for inhibition zones. The experiments were performed in duplicate and the average diameters of the zones of inhibition measured were recorded. According to the recorded data corresponding concentration of test compound with significant zone of inhibition was considered as MIC.

Cytotoxicity Assays

All the synthesized pyrimidines (BP_1-BP_{20}) are standard dissolved in DMSO, diluted with culture medium containing 0.1 % DMSO. The control cells were treated with culture medium containing 0.1 % DMSO. The compounds have been evaluated for their cytotoxicity against HT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines. Methotrexate was used as the reference standard. Data presented as mean ± SD (n=3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1 % DMSO. The control cells were treated with culture medium containing 0.1 % DMSO. NA- No Activity (i.e. IC_{50} > 200 µg/mL).

Results and Discussion

The pyrimidines synthesized showed antifungal activity with different MIC values against the tested organisms, but not comparable with that of the standard. It is also noticed that the pyrimidines tested showed more antifungal activity than the antibacterial activity. Among the compounds tested against *A.niger*, the compounds, BP_5 having a di fluorophenyl moiety, BP_6 is having a di chloro phenyl moiety, BP_7 having a 2-chloro-5-nitrophenyl moiety, BP_{14} having a bromofuran moiety and BP_{19} having a 4-pyridinyl moiety proved to be the most potent compounds with a MIC value of 16 µg/mL in each case. This was followed by the compounds, BP_2 (fluorophenyl moiety), BP_3 and BP_4 (chloro phenyl moieties), BP_8 and BP_9 (nitro phenyl moieties), BP_{17} (2-pyridinyl moiety) and BP_{20} (thienyl moiety) with a MIC value of 32 µg/mL in each case. Among the compounds tested against *C.tropicalis*, the compounds, BP_5 and BP_{20}, showed maximum activity with a MIC value of 16 µg/mL in each case. This was followed by

compounds, BP_1 (methyl phenyl), BP_6, BP_7 (2-chloro-5-nitrophenyl moiety), BP_{14} and BP_{19} with a MIC value of 32 µg/mL in each case.

Cytotoxic activity

The results clearly revealed that most of the pyrimidines possessed cytotoxic activity as evidenced by the IC_{50} values and is much higher than that of the chalcones indicating the positive contribution of pyrimidine nucleus in enhancing the cytotoxic activity. In fact, a number of anticancer drugs being used currently possessed pyrimidine nucleus as part of their structures. Of all the compounds tested against HT-29 cell lines, the compound BP_5 having a di fluoro phenyl moiety in its structure showed maximum activity with a IC_{50} value of 28 µg/mL. This is followed by compounds, BP_{20} having a thienyl moiety (IC_{50} 36 µg/mL), BP_2 and BP_6 having fluoro phenyl and di chloro phenyl moieties respectively (IC_{50} 42 µg/mL), BP_1 having a methyl phenyl moiety (IC_{50} 55 µg/mL) and BP_{14} having a bromofuran moiety (IC_{50} 56 µg/mL). The other compounds also showed activity but at a higher IC_{50} values. Among the compounds tested for cytotoxicity on MCF-7 cell lines, the compound BP_{14} showed maximum activity (IC_{50} 27 µg/mL). This was followed by compounds, BP_{20} (IC_{50} 28 µg/mL), BP_5 (IC_{50} 42 µg/mL) and BP_2 (IC_{50} 48 µg/mL). All the other compounds showed cytotoxicity at higher values. Among the compounds tested for cytotoxicity on DU-145 cell lines, the compounds, BP_{14} and BP_{20} showed maximum activity (IC_{50} 16 µg/mL). This was followed by compounds, BP_5 (IC_{50} 33 µg/mL), BP_{11} having a 3-nitro-4-methylphenyl moiety (IC_{50} 46 µg/mL), BP_1 (IC_{50} 52 µg/mL) and BP_6 (IC_{50} 56 µg/mL). It was also observed that among all the compounds tested on these three cell lines, most of the compounds showed maximum activity on prostate cancer cell lines (DU-145).

Conclusion

When the results of antifungal activity and acute toxicity of the chalcones compared with those of the pyrimidines, it is evident that the pyrimidines were more potent than the chalcones in most of the cases, even though some of the chalcones also showed comparable activity. Again the results indicated the importance of electron withdrawing groups in enhancing the activity. The fact that the chalcones as well as the pyrimidines showed maximum antifungal activity revealed that the conjugated carbonyl group in the case of chalcones and the amino pyrimidine moiety in the case of substituted pyrimidines is essential for the activity, the other substituent's being the same in both the cases. However, the contributing physicochemical properties need to be established by QSAR studies.

References

1. Vanderhaeghe, H. and Claesen, M., *Bull. Sci. Chim. Belg*., 1957, 66, 276.
2. Simson, M.M., *J. Am. Chem. Soc*., 1949, 71, 1470.
3. Baddar, F.G., Al-Hajjar, F.H and El-Rayyes, N.R., *J. Het. Chem*., 1971, 13, 257.
4. Brown, D.J., in: *Comprehensive Heterocyclic*

Chemistry, *Pergman*, 1984, 3, 57.

5. Al-Hajjar, F.H. and Sabir, S.S., *J. Het. Chem*., 1982, 19, 1087.

6. Reddy, G.S.I., Hobgood, R.T. and Gold Stein, J.H., *J. Am. Chem. Soc*., 1962, 84, 336.

7. El-Rayyes, N. R. and Al-Hajjar, F.H., J. *Het. Chem*., 1977, 14, 367.

8. Rice, J.M., Dudek, G.O. and Barber, M., *J. Am. Chem. Soc*., 1965, 87, 4569.

9. Nishiwaki, T., *Tetrahedron*, 1966, 22, 3117.

10. Spitller, G., in: *Physical Methods in Hetrocyclic Chemistry*, *Acadamic Press*, *New York*, 1971, 3, 286.

11. Hitchings, G.H., Ann. N.Y. Acad. Sci., 1961, 23, 700.

12. Patel, V.G. and Patel, J.C., *Ultra Scientist of Physical Sci*., 2004, 16, 111.

13. Mishra, B.K., Mishra, R. and Harinarayana Moorthy, N.S., *Trends in Applied Sciences Research*, 2008, 3, 203.

14. Bodke, Y. and Sangapure, S.S., J. Indian Chem. Soc., 2003, 80, 187.

15. Murthy, Y.L.N., Rani, N., Ellaiah, P. and Bhavani devi, R., *Het. Comm*., 2005, 11, 189.

16. Kudari, S.M., Munera, W. and Beede, S.M., *Oriental J. Chem*., 1996, 12, 167.

17. Cottam, H.B., Wasson, D.B., Shih, H.C., Raychaudhary, A., Pasquale, G.D. and Carson, D.A., *J. Med. Chem*., 1993, 36, 3424.

18. Bruni, F., Costazo, A., Selleri, S., Guerrini, G., Fantozzi, R., Pirisino, R. and Brunelleschi, S., *J. Pharm. Sci*., 1993, 82, 480.

19. Vidal, A., Ferrandiz, M.L., Ubeda, A., Alarcon, A.A., Arques, J.S. and Alcarz, M.J., *J. Pharm. Pharmacol*., 2001, 53, 1379.

20. Bruni, O., Brullo, C., Ranise, A., Schenone, S., Bondavalls, S., Barvocelli, E., Ballabeni, V., Chivarani, M., Tognolini, M. and Jmpicciatore, M., *Bioorg. Med. Chem. Lett*., 2001, 11, 1397.

21. Ferri, P.F., Ubeda, A., Guillen, J., Lasri, J., Rosende, M.E.G., Akssir, M. and Arques, J.S., *Eur. J. Med. Chem*., 2003, 38, 289.

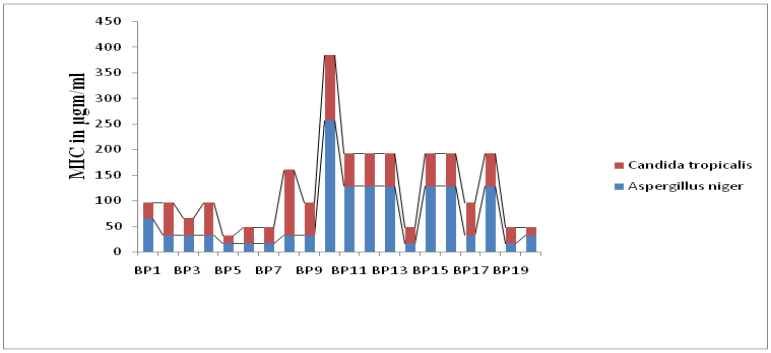
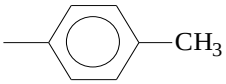
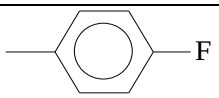
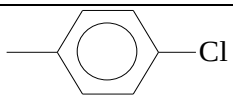
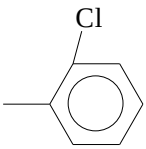
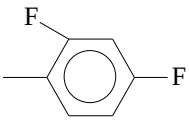
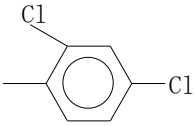
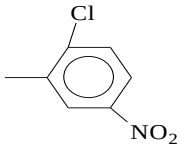
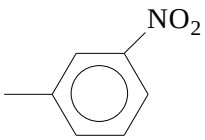
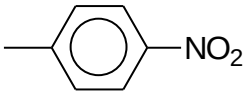
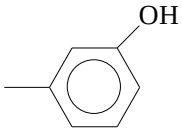
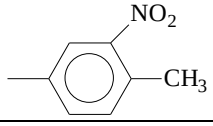
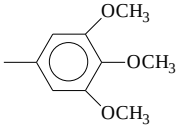


Figure 3: Antifungal activity of pyrimidines (BP_1 to BP_{20}) (Expressed as MIC in µg/mL)

Table 1: Physical characterization data of 2,4,6-trisubstituted pyrimidines (BP_1 BP_{20})

Compound	Ar	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (^oC)	Yield %
BP_1		$C_{16}H_{15}N_3O$	265	121	63
BP_2		$C_{15}H_{12}FN_3O$	269	136	78
BP_3		$C_{15}H_{12}ClN_3O$	285	129	73
BP_4		$C_{15}H_{12}ClN_3O$	285	122	75
BP_5		$C_{15}H_{11}F_2N_3O$	287	143	72
BP_6		$C_{15}H_{11}Cl_2N_3O$	319	132	69
BP_7		$C_{15}H_{11}ClN_4O_3$	330	137	57
BP_8		$C_{15}H_{12}N_4O_3$	296	176	64
BP_9		$C_{15}H_{12}N_4O_3$	296	184	72
BP_{10}		$C_{15}H_{13}N_3O_2$	267	210	75
BP_{11}		$C_{16}H_{14}N_4O_3$	310	168	66
BP_{12}		$C_{18}H_{19}N_3O_4$	341	193	77

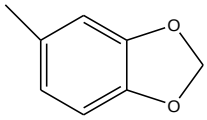
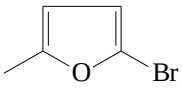
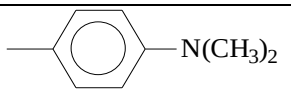
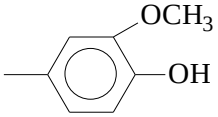
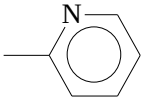
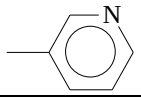
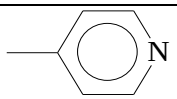
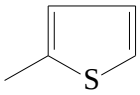
BP_{13}		$C_{16}H_{13}N_3O_3$	295	153	62
BP_{14}		$C_{13}H_{10}BrN_3O_2$	320	147	67
BP_{15}		$C_{17}H_{18}N_4O$	294	126	79
BP_{16}		$C_{16}H_{15}N_3O_3$	297	205	68
BP_{17}		$C_{14}H_{12}N_4O$	252	117	56
BP_{18}		$C_{14}H_{12}N_4O$	252	123	62
BP_{19}		$C_{14}H_{12}N_4O$	252	135	67
BP_{20}		$C_{13}H_{11}N_3OS$	257	112	63

Table 2: IR spectral data (K Br disc) of 2,4,6-trisubstituted pyrimidines (BP_1-BP_{20})

Compound	Position of absorption band (cm^{-1})
BP_1	3475, 3329 (NH_2), 1585 (C=N), 1505 (C=C), 1395 (C-N) and 1085 (C-O-C).
BP_2	3483, 3296 (NH_2), 1625 (C=N), 1509 (C=C),1399 (C-N), 1092 (C-O-C) and 831 (C-F)
BP_3	3425, 3329 (NH_2), 1595 (C=N), 1502 (C=C), 1384 (C-N), 778 (C-Cl) and 1110 (C-OC)
BP_4	3427, 3332 (NH_2), 1596 (C=N), 1510 (C=C), 1365 (C-N), 1095 (C-O-C) and 805 (C-Cl)
BP_5	,3420, 3355 (NH_2), 1612 (C=N), 1501 (C=C),1382 (C-N), 1088 (C-O-C) and 844 (C-F)
BP_6	3432, 3359 (NH_2), 1593 (C=N), 1502 (C=C), 1382 (C-N), 1085 (C-O-C) and 805 (C-Cl)
BP_7	3428, 3349 (NH_2), 1588 (C=N), 1520 (N=O, asymmetric), 1505 (C=C), 1382 (C-N), 1340 (N=O, symmetric), 1101 (C-O-C) and 781 (C-Cl)
BP_8	3420, 3335 (NH_2), 1580 (C=N), 1522 (N=O, asymmetric), 1501 (C=C), 1385 (C-N), 1345 (N=O, symmetric) and 1092 (C-O-C)
BP_9	3418, 3330 (NH_2), 1586 (C=N), 1515 (N=O, asymmetric), 1506 (C=C), 1380 (C-N), 1338 (N=O, symmetric) and 1085 (C-O-C)
BP_{10}	3520 (OH), 3428, 3355 (NH_2), 1653 (C=N), 1528 (C-N), 1502 (C=C) and 1083 (C-O-C)
BP_{11}	3423, 3348 (NH_2), 1642 (C=N), 1548 (N=O, asymmetric), 1510 (C=C), 1380 (C-N), 1338 (N=O, symmetric) and 1092 (C-O-C)

BP_{12}	3420, 3350 (NH_2), 1648 (C=N), 1505 (C=C), 1365 (C-N), 1225 (-O-CH_3) and 1088 (C-O-C)
BP_{13}	3430, 3370 (NH_2), 1592 (C=N), 1502 (C=C), 1370 (C-N), 1232 (-O-CH_2-O-) and 1095 (C-O-C)
BP_{14}	3410, 3360 (NH_2), 1602 (C=N), 1505 (C=C), 1340 (C-N), 1085 (C-O-C) and 790 (C-Br)
BP_{15}	3412, 3332 (NH_2), 1608 (C=N), 1509 (C=C), 1390 (C-N), 1175 (-N-$(CH_3)_2$) and 1080 (C-O-C)
BP_{16}	3540 (O-H), 3415, 3382 (NH_2), 1598 (C=N), 1502 (C=C), 1378 (C-N), 1234 (-O-CH_3) and 1090 (C-O-C)
BP_{17}	3423, 3365 (NH_2), 1602 (C=N), 1510 (C=C), 1390 (C-N) and 1086 (C-O-C)
BP_{18}	3420, 3362 (NH_2), 1599 (C=N), 1506 (C=C), 1382 (C-N) and 1092 (C-O-C)
BP_{19}	3420, 3352 (NH_2), 1606 (C=N), 1508 (C=C), 1388 (C-N) and 1082 (C-O-C)
BP_{20}	3422, 3356 (NH_2), 1605 (C=N), 1503 (C=C), 1386 (C-N), 108 (C-O-C) and 644 (C-S)

Table 3: ^{1}H NMR spectral data of 2, 4, 6-trisubstituted pyrimidines ($BP_1 – BP_{20}$)

Compound	**Chemical shift (δ) in ppm**
BP_1	2.40; 2.65 (each 3H, s, 2XAr-CH_3), 7.22 (1H, s, C-5-H), 6.61 (2H, s, C-2-NH_2), 7.20-8.10 (6H, Ar-H).
BP_2	2.45 (3H, s, Ar-CH_3), 7.05 (1H, s, C-5-H), 5.19 (2H, s, C-2-NH_2), 7.20-8.09 (6H, Ar-H).
BP_3	2.46 (3H, s, Ar-CH_3), 7.25 (1H, s, C-5-H), 6.65 (2H, s, C-2-NH_2), 7.22-8.08 (6H, Ar-H).
BP_4	2.40 (3H, s, Ar-CH_3), 7.12 (1H, s, C-5-H), 6.72 (2H, s, C-2-NH_2), 6.95-7.60 (6H, Ar-H).
BP_5	2.43 (3H, s, Ar-CH_3), 7.08 (1H, s, C-5-H), 6.30 (2H, s, C-2-NH_2), 6.98-8.12 (5H, Ar-H).
BP_6	2.48 (3H, s, Ar-CH_3), 7.15 (1H, s, C-5-H), 6.20 (2H, s, C-2-NH_2),7.05-7.95 (5H, Ar-H).
BP_7	2.43 (3H, s, Ar-CH_3), 7.09 (1H, s, C-5-H), 6.12 (2H, s, C-2-NH_2), 6.98-8.10 (5H, Ar-H).
BP_8	2.40 (3H, s, Ar-CH_3), 7.30 (1H, s, C-5-H), 6.80 (2H, s, C-2-NH_2), 7.48-8.60 (6H, Ar-H).
BP_9	2.45 (3H, s, Ar-CH_3), 7.18 (1H, s,C-5-H), 6.25 (2H, s, C-2-NH_2), 7.25-8.20 (6H, Ar-H).
BP_{10}	2.40 (3H, s, Ar-CH_3), 7.25 (1H, s,C-5-H), 6.30 (2H, s, C-2-NH_2), 7.15-7.80 (6H, Ar-H), 6.85 (1H, s , Ar-OH).

BP_{11}	2.70 and 2.50 (each 3H, s, 2XAr-CH_3), 7.30 (1H, s,C-5-H), 6.70 (2H, s, C-2-NH_2), 7.45-8.78 (5H, Ar-H)
BP_{12}	2.45 (3H, s, Ar-CH_3), 7.22 (1H, s,C-5-H), 6.60 (2H, s, C-2-NH_2), 7.30-7.50 (4H, Ar-H), 3.70 (3H, s, Ar-OCH_3), 3.88 (6H, s, 2XAr-OCH_3)
BP_{13}	2.40 (3H, s, Ar-CH_3), 7.25 (1H, s,C-5-H), 6.40 (2H, s, C-2-NH_2), 6.10 (2H, s, O-CH_2-O), 7.21-7.85 (5H, Ar-H)
BP_{14}	2.50 (3H, s, Ar-CH_3), 7.10 (1H, s,C-5-H), 5.80 (2H, s, C-2-NH_2), 6.80-7.30 (4H, Ar-H
BP_{15}	2.80 (3H, s, Ar-CH_3), 3.20 (6H, s, N-$(CH_3)_2$, 7.20 (1H, s,C-5-H), 5.45 (2H, s, C-2-NH_2), 6.70-8.20 (6H, Ar-H)
BP_{16}	2.45 (3H, s, Ar-CH_3), 7.20 (1H, s,C-5-H), 5.85 (2H, s, C-2-NH_2), 7.15-7.90 (5H, Ar-H), 6.95 (1H, s, Ar-OH), 3.80 (3H, s, Ar-O-CH_3)
BP_{17}	2.40 (3H, s, Ar-CH_3), 7.15 (1H, s,C-5-H), 6.20 (2H, s, C-2-NH_2), 7.10-8.15 (6H, Ar-H)
BP_{18}	2.70 (3H, s, Ar-CH_3), 7.25 (1H, s,C-5-H), 5.30 (2H, s, C-2-NH_2), 6.75-8.90 (6H, Ar-H)
BP_{19}	2.58 (3H, s, Ar-CH_3), 7.20 (1H, s,C-5-H), 5.50 (2H, s, C-2-NH_2), 6.95-8.68 (6H, Ar-H)
BP_{20}	2.68 (3H, s, Ar-CH_3), 7.20 (1H, s,C-5-H), 5.34 (2H, s, C-2-NH_2), 6.60-7.80 (5H, Ar-H)

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