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# Synthesis, screening and QSAR studies of 3-formyl-2-thioxo-1,2,3,4-tetrahydropyrimidine analogues as antibacterial agents

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**ABSTRACT**

5-acyl-6-methyl-4-substituted-2-thioxo-1,2,3,4-tetrahydropyrimidines **(1)** were synthesized by cyclocondensation reaction between appropriate aldehyde, acetoacetate and thiourea in presence of aluminium chloride and hydrochloric acid which upon treatment with dimethylformamide and phosphorous oxychloride furnish the title compounds **(2a-j)**. The structures of all title compounds have been confirmed on the basis of their analytical, IR and NMR spectral data. The title compounds have been tested for antibacterial activity against *Staphylococcus aureus*. A quantitative structure activity relationship study was made using various descriptors. Several statistical expressions were developed using stepwise multiple linear regression analysis. The best quantitative structure activity relationship model was further cross validated. The study revealed that electronic property (dipoleX) and spatial descriptor (DCASA) both contributes negatively which suggest that minimizing both the dipole moment on X-axis and absolute difference in charge-weighted area may lead to better antibacterial compound from this series.

**Key words:** 2-thioxo-1,2,3,4-tetrahydropyrimidines, Antibacterial, QSAR**.**

## INTRODUCTION

The resistance of common pathogens to standard antibiotic therapy is rapidly becoming a major health problem throughout the world. The resistance of multidrug-resistant gram-positive bacteria is increasing and infections caused by *Staphylococcus aureus*, *enterococci* and *pneumococci* are particularly problematic[1]. There is a real perceived need for the discovery of new compounds endowed with antibacterial property.

QSAR studies of antimicrobial activity represent an emerging and exceptionally important topic in the area of computer-aided drug design. Although the demand for 'insilico' discovery is clear in all area of human therapeutics, the field of anti-infective drugs has a particular need for computational solutions enabling rapid identification of novel therapeutic leads. As a result, there is an urge for new antimicrobial driven by critical situation, such as increased prevalence of multidrug-resistant bacteria and the emergence of deadly infectious diseases.

In recent years, substituted 2-oxo/thioxo-1,2,3,4-tetrahydropyrimidines received significant attention owing to their diverse range of biological properties such as calcium channel modulator[2], 1-adrenoreceptor selective antagonist[3], HIV gpl20-$CD_4$ inhibition[4], antiviral[5], anticancer with mitotic kinesin inhibition[6], inhibitor of Walker carcinosarcoma[7], oral antihypertensive[8], blood platelet aggregation inhibition[9], useful for the treatment of benign prostatic hyperplasia[10], anti-inflammatory, antifungal and antibacterial[11]. The presence of several interacting functional groups in these compounds also determines their great synthetic potential[12].

In the present paper we describe the synthesis, screening and QSAR studies to investigate the relationship between the various physicochemical parameters and antibacterial activity of synthesized 3-formyl derivatives of 5-acyl-6-methyl-4-substituted-2-thioxo-1,2,3,4-tetrahydropyrimidines.

## MATERIAL AND METHODS

Melting points of the synthesized compounds were determined in open capillary tubes are therefore uncorrected. The structures of the title compounds were established on the basis of elemental analysis and spectral data. The IR spectra were recorded on JASCO FTIR 4100 spectrophotometer. $^1$H NMR spectra were recorded on Varian NMR 400 MHz spectrometer using $CDCl_3$/ DMSO-$d_6$ as solvent with TMS as an internal standard. Purity of the synthesized compounds was checked by silica gel-G plate using benzene and ethyl acetate as developer.

**General procedure for the synthesis of 5-acyl-6-methyl-4-substituted-2-thioxo-1,2,3,4-tetrahydropyrimidines(1)**

These compounds were synthesized by the reported cyclocondensation reaction[13,14] between aldehyde, acetoacetate and thiourea. The mixture of appropriate aldehyde (0.02 mole), acetoacetate (0.02 mole), thiourea (0.03 mole), aluminium chloride (0.01 mole), conc. hydrochloric acid 2 drops in methanol were refluxed for 4 h. The solid thus separated on cooling was filtered, washed with cold methanol, dried and recrystallized from methanol.

**General procedure for the synthesis of 3-formyl derivatives of 5-acyl-6-methyl-4-substituted-2-thioxo-1,2,3,4-tetrahydropyrimidines(2a-j)**

To a suspension of respective 5-acyl-6-methyl-4-substituted-2-thioxo-1,2,3,4-tetrahydropyrimidines (0.02 mole) in 20 mL of dry dimethylformamide, phosphorous oxychloride (0.02 mole) was added in ice-bath. The resulting solution was heated at 70°C and kept there for 40 minutes and then was poured into 150 ml of ice-water to yield the solid product. The solid product thus separated was filtered, washed with cold water, dried and recrystallized from ethanol.

**Antibacterial activity**

Antibacterial activity of these ten compounds was tested *in vitro* against gram-positive bacteria *Staphylococcus aureus* (NCIM-2079) by the *Cup-plate agar diffusion* method, using dimethyl sulfoxide as solvent and trimethoprim as standard drug. Further minimum inhibitory concentration (MIC) of all these compounds was determined by double dilution method[15]. The biological data minimum inhibitory concentration (MIC) in mg/mL were converted to negative logarithmic dose in moles (pMIC) for QSAR analysis.

The series was subjected to QSAR analysis using MOE 2006.08 running on P-IV processor. Structures of all the compounds were sketched using builder module of the programme. These structures were then subjected to energy minimization using Hamiltonian force field molecular mechanics-MMFF 94X by fixing root mean square (RMS) gradient as 0.01 kcal/mol A°.The descriptor values for all the molecules were calculated using "compute descriptor" module of the programme. All the calculated descriptors were considered as independent variable and biological activity (pMIC) as dependent variable. Stepwise multiple linear regression analysis method was used to perform QSAR analysis to generate several models. The best model was selected on the basis of various statistical parameters such as squared correlation coefficient ($r^2$), standard error of estimation (SE), sequential Fischer test (F). Quality and predictability of model was estimated from the cross validated squared correlation coefficient ($q^2$)[16].

## RESULTS AND DISCUSSION

The purity and homogeneity of all the title compounds were confirmed by their sharp melting points and TLC. In all cases these compounds were obtained in solid state and the yields varied from maximum 82% to minimum 40%. The synthesized compounds were subjected to physico-chemical characterization and elemental analysis (Table 1). The structures of these compounds were confirmed by C, H and N analytical data, IR and $^1$H NMR spectral data (Table 2). Antimicrobial activity data against *Staphylococcus aureus* minimum inhibitory concentration (MIC) in mg/ml was converted to negative logarithmic dose in moles (pMIC) for QSAR

**Table 1: Characterization data of the title compounds (2a – j)**

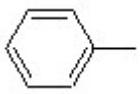
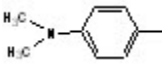
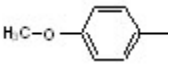
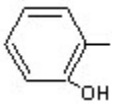
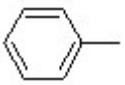
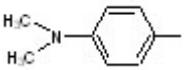
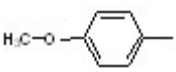
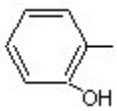
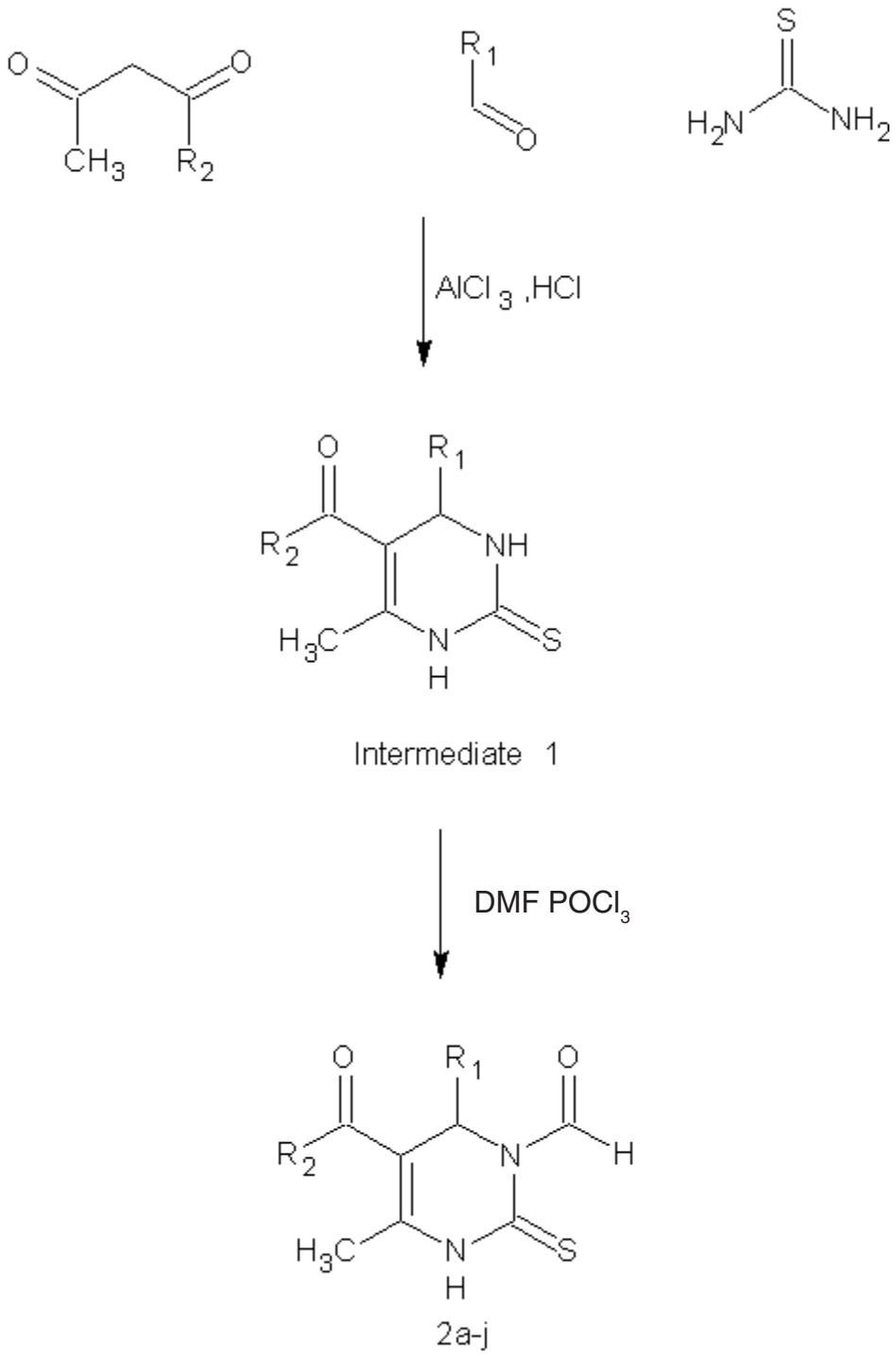
| Comp. | $R_1$ | $R_2$ | Mol. Formula | Yield (%) | m.p. (°C) | Analysis Found (Cal.) % C | H | N |
|---|---|---|---|---|---|---|---|---|
| 2a |  | $OC_2H_5$ | $C_{15}H_{16}N_2O_3S$ | 81.66 | 160 | 59.11 (59.19) | 5.20 (5.30) | 9.12 (9.20) |
| 2b | H | $OC_2H_5$ | $C_9H_{12}N_2O_3S$ | 48.61 | 226 | 47.26 (47.35) | 5.22 (5.30) | 12.18 (12.27) |
| 2c |  | $OC_2H_5$ | $C_{17}H_{21}N_3O_3S$ | 52.63 | 140 | 58.67 (58.77) | 6.01 (6.09) | 12.02 (12.09) |
| 2d |  | $OC_2H_5$ | $C_{16}H_{18}N_2O_4S$ | 61.81 | 70 | 57.36 (57.47) | 5.35 (5.43) | 8.29 (8.38) |
| 2e |  | $OC_2H_5$ | $C_{15}H_{16}N_2O_4S$ | 68.25 | 110 | 56.15 (56.24) | 4.96 (5.03) | 8.65 (8.74) |
| 2f |  | $OCH_3$ | $C_{14}H_{14}N_2O_3S$ | 78.94 | 170 | 57.83 (57.92) | 4.77 (4.86) | 9.57 (9.65) |
| 2g | H | $OCH_3$ | $C_8H_{10}N_2O_3S$ | 40.81 | 152 | 44.75 (44.85) | 4.62 (4.70) | 13.01 (13.08) |
| 2h |  | $OCH_3$ | $C_{16}H_{19}N_3O_3S$ | 41.48 | 70 | 57.55 (57.64) | 5.65 (5.74) | 12.51 (12.60) |
| 2i |  | $OCH_3$ | $C_{15}H_{16}N_2O_4S$ | 47.58 | 154 | 56.25 (56.24) | 4.95 (5.03) | 8.65 (8.74) |
| 2j |  | $OCH_3$ | $C_{14}H_{14}N_2O_4S$ | 50.00 | 160 | 54.80 (54.89) | 4.51 (4.61) | 9.05 (9.14) |

**Table 2: IR and $^1$H NMR spectral data of the title compounds (2a – j)**

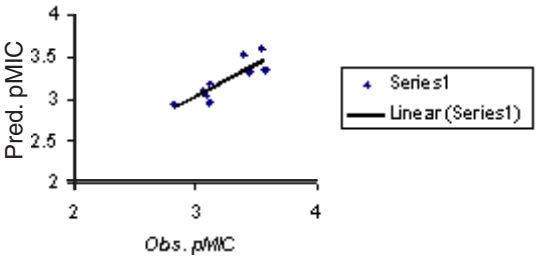
| Compound | IR $cm^{-1}$ | $^1$H NMR δ ppm |
|---|---|---|
| 2a | 3240,3140 (N-H), 2970 (C-H), 1720 (C=O), 1700 (C=O), 1520 (C=S) | 1.11 (t, 3H, ethyl $CH_3$), 2.30 (s, 3H, $C_6$-$CH_3$), 3.99 (q, 2H, $OCH_2$), 6.55 (s, 1H, methine CH), 7.09-7.74 (m, 5H, Ph), 9.19 (s, 1H, formyl CH), 8.56 (s, 1H, NH) |
| 2b | 3260, 3130 (N-H), 2960 (C-H), 1715 (C=O), 1690 (C=O), 1525 (C=S) | 1.22 (t, 3H, ethyl $CH_3$), 2.46 (s, 3H, $C_6$-$CH_3$), 4.10 (q, 2H, $OCH_2$), 4.71 (s, 2H, methylene $CH_2$), 9.09 (s, 1H, formyl CH), 8.86 (s, 1H, NH) |
| 2c | 3245, 3140 (N-H), 2980 (C-H), 1710 (C=O), 1695 (C=O), 1515(C=S) | 1.11 (t, 3H, ethyl $CH_3$), 2.30 (s, 3H, $C_6$-$CH_3$), 4.01 (q, 2H, $OCH_2$), 6.55 (s, 1H, methine CH), 6.47-7.56 (m, 4H, Ph), 2.83 (s, 6H, $N(CH_3)_2$), 9.19 (s, 1H, formyl CH), 8.56 (s, 1H, NH) |
| 2d | 3250,3145 (N-H), 2975 (C-H), 1715 (C=O), 1695 (C=O), 1510 (C=S) | 1.12 (t, 3H, ethyl $CH_3$), 2.31 (s, 3H, $C_6$-$CH_3$), 3.99 (q, 2H, $OCH_2$), 6.54 (s, 1H, methine CH), 7.00-7.63 (m, 4H, Ph), 3.60 (s, 3H, $OCH_3$), 9.20 (s, 1H, formyl CH), 8.58 (s, 1H, NH) |
| 2e | 3250,3100 (N-H), 2980 (C-H), 1715 (C=O), 1680 (C=O), 1520 (C=S) | 1.13 (t, 3H, ethyl $CH_3$), 2.30 (s, 3H, $C_6$-$CH_3$), 3.99 (q, 2H, $OCH_2$), 6.43 (s, 1H, methine CH), 6.84-7.55 (m, 4H, Ph), 6.39 (s, 1H, Ar-OH), 9.19 (s, 1H, formyl CH), 8.56 (s, 1H, NH) |
| 2f | 3240,3140 (N-H), 2970 (C-H), 1720 (C=O), 1690 (C=O), 1510 (C=S) | 3.71 (s, 3H, $COOCH_3$), 2.29 (s, 3H, $C_6$-$CH_3$), 6.55 (s, 1H, methine CH), 7.10-7.74 (m, 5H, Ph), 9.16 (s, 1H, formyl CH), 8.54 (s, 1H, NH) |
| 2g | 3260,3125 (N-H), 2965 (C-H), 1710 (C=O), 1690 (C=O), 1515 (C=S) | 3.72 (s, 3H, $COOCH_3$), 2.46 (s, 3H, $C_6$-$CH_3$), 4.71 (s, 2H, methylene $CH_2$), 9.09 (s, 1H, formyl CH), 8.86 (s, 1H, NH) |
| 2h | 3245,3135 (N-H), 2985 (C-H), 1715 (C=O), 1690 (C=O), 1510 (C=S) | 3.71 (s, 3H, $COOCH_3$), 2.30 (s, 3H, $C_6$-$CH_3$), 6.55 (s, 1H, methine CH), 6.47-7.56 (m, 4H, Ph), 2.83 (s, 6H, $N(CH_3)_2$), 9.19 (s, 1H, formyl CH), 8.57 (s, 1H, NH) |
| 2i | 3220,3100 (N-H), 2980 (C-H), 1705 (C=O), 1690 (C=O), 1510 (C=S) | 3.71 (s, 3H, $COOCH_3$), 2.30 (s, 3H, $C_6$-$CH_3$), 6.55 (s, 1H, methine CH), 6.92-7.63 (m, 4H, Ph), 3.61 (s, 3H, $OCH_3$), 9.18 (s, 1H, formyl CH), 8.56 (s, 1H, NH) |
| 2j | 3250,3100 (N-H), 2980 (C-H), 1715 (C=O), 1680 (C=O), 1520 (C=S) | 3.71(s, 3H, $COOCH_3$), 2.13 (s, 3H, $C_6$-$CH_3$), 6.50 (s, 1H, methine CH), 6.85-7.25 (m, 4H, Ph), 6.62 (s, 1H, Ar-OH), 9.19 (s, 1H, formyl CH), 8.56 (s, 1H, NH) |




**Synthesis scheme**

**Table 3: Antibacterial activity of the title compounds (2a – j) on *S. aureus***

| Comp. | Minimum inhibitory concentration (MIC) in µg/ml | pMIC |
|---|---|---|
| 2a | 125 | 3.3865 |
| 2b | 62 | 3.5661 |
| 2c | 125 | 3.4440 |
| 2d | 500 | 2.8253 |
| 2e | 250 | 3.1077 |
| 2f | 250 | 3.0650 |
| 2g | 62 | 3.5385 |
| 2h | 500 | 2.8240 |
| 2i | 250 | 3.1077 |
| 2j | 250 | 3.0883 |




Obs. pMIC: Observed pMIC, Pred. pMIC: Predicted pMIC,
$y=0.7808x + 0.6982$, $r^2=0.7785$.

**Fig. 1: Plot between observed vs. predicted pMIC values for model - 3**

**Table 4: Calculated molecular descriptors of the title compounds (2a – j)**

| Comp. | [A]dipoleZ | [B]BCUT_SLOGP_3 | [C]opr_brigid | [D]dipoleX | [E]ASA+ | [F]CASA+ | [G]DCASA |
|---|---|---|---|---|---|---|---|
| 2a | 0.0975 | 2.5521 | 12.0000 | -1.1835 | 169.7962 | 671.7139 | 35.2657 |
| 2b | -0.2632 | 2.4226 | 6.0000 | -1.1793 | 103.8552 | 318.0046 | 82.0116 |
| 2c | -0.4801 | 2.5592 | 12.0000 | -0.9186 | 163.2914 | 758.3255 | 15.0785 |
| 2d | -1.0267 | 2.5575 | 12.0000 | -0.6505 | 163.4152 | 681.1144 | 45.7969 |
| 2e | -0.5082 | 2.5693 | 12.0000 | -0.8056 | 178.6508 | 774.9872 | 22.7961 |
| 2f | -0.8152 | 2.5497 | 12.0000 | -0.7631 | 186.2613 | 736.8497 | 30.6865 |
| 2g | -0.2659 | 2.4079 | 6.0000 | -1.1799 | 127.9898 | 391.9047 | 15.0271 |
| 2h | -0.4809 | 2.5572 | 12.0000 | -0.9221 | 189.3483 | 879.3336 | 124.3562 |
| 2i | -1.0276 | 2.5554 | 12.0000 | -0.6612 | 187.5038 | 781.5159 | 44.0332 |
| 2j | -0.5056 | 2.5675 | 12.0000 | -0.8177 | 201.1047 | 872.3921 | 60.9024 |

A: Dipole moment (Z), B: LogP BCUT (3/3), C: Oprea rigid bond count, D: Dipole moment (X), E: Positive accessible surface area, F: Charge-weighted positive surface area, G: Absolute difference in charge-weighted areas.

**Table 5: Observed (obs.), Predicted (pred.) pMIC and residual values for model - 3**

| Compound | pMIC observed | pMIC predicted | Residuals |
|---|---|---|---|
| 2a | 3.3865 | 3.5265 | -0.1400 |
| 2b | 3.5661 | 3.3359 | 0.2070 |
| 2c | 3.4440 | 3.3203 | 0.1236 |
| 2d | 2.8253 | 2.9331 | -0.1078 |
| 2e | 3.1077 | 3.1754 | -0.0677 |
| 2f | 3.0650 | 3.1034 | -0.0384 |
| 2g | 3.5385 | 3.5934 | -0.0549 |
| 2h | 2.8240 | 2.9426 | -0.1186 |
| 2i | 3.1077 | 2.9505 | 0.1572 |
| 2j | 3.0883 | 3.0488 | 0.0395 |

analysis (Table 3). Values of descriptors (Table 4) which are significant in model are showing high correlation with biological activity. Performing stepwise multiple linear regression analysis results in several equations out of that five are found to be statistically significant QSAR models.

pMIC 9.20811 - 2.30344 (±1.0890) *BCUT_SLOGP_3 + 0.35156(±0.1880)*dipoleZ, n=10, $r^2$=0.64690, $q^2$=0.482103, SE=0.1832, F=6.48(Model-1).

pMIC 3.97612 - 0.05526(±0.0265)*opr_brigid + 0.34877(±0.1902)*dipoleZ,n=10, $r^2$=0.64467, $q^2$=0.470644, SE=0.1847, F=6.35 (Model-2).

pMIC 2.41365 - 1.04425(±0.2253)*dipoleX - 0.00349 (±0.0014)* DCASA, n=10, $r^2$=0.79353, $q^2$=0.462507, SE=0.1408, F=13.44(Model-3).

pMIC 4.20619 + 0.33855(±0.1810)*dipoleZ - 0.00498(±0.0021)*ASA+ n=10, $r^2$=0.67760, $q^2$=0.450981, SE=0.1760, F=7.36(Model-4).

pMIC 3.93072 + 0.34224(±0.0.1764)*dipoleZ - 0.00081 (±0.0003)*CASA+, n=10, $r^2$=0.68956, $q^2$=0.474811, SE=0.1727, F=7.77(Model-5).

Out of the five models, model-3 was selected on the basis of statistical criteria; $r^2$ =0.79353, SE=0.14080 and F=13.44. The internal predictivity of the model was assessed by cross-validated squared correlation coefficient ($q^2$ =0.462507), which shows good correlation between predicted activity and observed activity (Table 5 and Fig.1). Correlation matrix shows poor correlation between descriptors (Table 6).

**Table 6: Correlation matrix**

| | pMIC | dipoleZ | BCUT_SLOGP_3 | opr_brigid | dipoleX | ASA+ | CASA+ | DCASA |
|---|---|---|---|---|---|---|---|---|
| pMIC | 1.0000 | | | | | | | |
| dipoleZ | 0.6508 | 1.0000 | | | | | | |
| BCUT_SLOGP_3 | 0.6872 | 0.3860 | 1.0000 | | | | | |
| opr_brigid | 0.6885 | 0.3937 | 0.9934 | 1.0000 | | | | |
| dipoleX | 0.7777 | 0.9161 | 0.6956 | 0.6873 | 1.0000 | | | |
| ASA+ | 0.7186 | 0.3916 | 0.8904 | 0.8976 | 0.6750 | 1.0000 | | |
| CASA+ | 0.7230 | 0.3769 | 0.9344 | 0.9283 | 0.6703 | 0.9632 | 1.0000 | |
| DCASA | 0.3991 | 0.0209 | 0.0103 | 0.0143 | 0.0448 | 0.0729 | 0.1500 | 1.0000 |

It is evident from the QSAR studies that in model-3, electronic descriptor (dipoleX) and spatial descriptor (DCASA) are responsible for the activity and both contributes negatively to biological activity, which indicates that minimizing both the dipole moment on X axis and absolute difference in charge-weighted area may lead to better antibacterial compound from this series.

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## REFERENCES


1. Neu, H.C., *Science*, **257**: 1064 (1992).
2. Kappe, C.O., *Molecules*, **3**: 1 (1998).
3. Barrow, J.C., Glass, K.L., Selnick, H.G., Freidinger, R.M., Chang, R.S.L., O'Malley, S.S. and Woyden, C., *Bioorg. Med. Chem. Lett.*, **10**(17): 1917 (2000).
4. Patil, A.D., Kumar, N.V., Kokke, W.C., Bean, M.F., Freyer, A.J., Debrose, C. Mai S., Trunch, A., Falkner, D.J., Carte, B., Breen, A.L., Hertzberg, R.P., Johnston, R.K., Westely, J.W. and Ports B.C.M., *J. Org. Chem.*, **6**: **1182** (1995).
5. Hurst, E.W. and Hull, R., *J. Med. Pharm. Chem.*, **3**: 215 (1961).
6. Meyer, T.V., Kapoor, T.M., Haggarty, S.J., King, R.W., Schreiber, S.L. and Mitchison, T.J., *Science*, **286**: 71 (1991).
7. Kumasars, K., Valena, A., Dubers, G., Uldrikis, J. and Ziderman, A., *Biokhimiya*, **36**: 1201 (1971).
8. Kappe, C.O., *Acc. Chem. Res.*, **33**: 879 (2000).
9. Tozkoparan, B., Akgun, H., Ertan, M., Sara, Y. and Ertekin, N., *Arch. Pharm.*, **328**: 169 (1995).
10. Nagarathnam, D., Wetzel, J.M., Miao, S.W., Marzabad, M.R., Chiu, G., Wang, W.C., Hong, X., Fang, J., Forry, C., Branchek, T.A., Heydora, W.E., Chang, R.S.L., Broten, T., Schora, T. and Gluchowski, C., *J. Med. Chem.*, **41**: 5320 (1998).
11. Kappe, C.O., *Eur. J. Med. Chem.*, **35**: 1043 (2000).
12. Kappe, C.O., *Tetrahedron*, **49**: 6937 (1993).
13. Russowsky, D., Lopes, F.A., da Silva, V.S.S., Canto, K.F.S., D'Oca, M.G.M. and Godoi, M.N., *J. Braz Chem. Soc.*, **15**(2): 165 (2004).
14. Kumar, S., Saini, A. and Sandhu, J.S., *Indian J. Chem.*, **43**B: 1485 (2004).
15. Kirven, L.A. and Thornsberry, C., *Antimicrob. Agents Chemother.*, **10**: 340 (1978).
16. Kubinyi H., *QSAR: Hansch Analysis and Related Approaches*, Weinheim, New York, 91 (1993).