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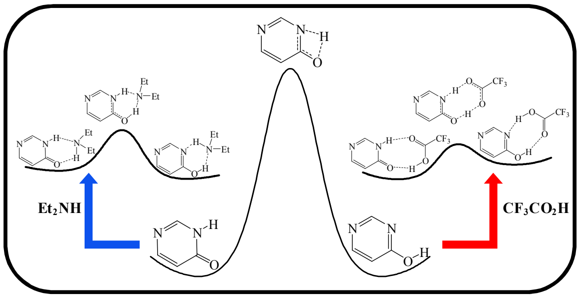

Relative stabilities of tautomers and tautomeric barriers are extensively modulated by use of carboxylic acids and amine bases

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External control over tautomeric distribution and inter-conversion: New insights into the realm of catalyzed tautomerization

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The study shows that relative stabilities of two tautomeric forms for four isomeric amide-imidic acid pairs can be extensively altered by use of a simple carboxylic acid and an amine base. Further, the acid and base act as highly efficient catalysts by drastically reducing the barriers for tautomeric transformations. It is also shown that both the relative stabilities and barrier heights can be precisely modulated by changing the carboxylic acids and amine bases. The extents of both selective stabilization and barrier lowering are found to show linear dependence on the strength of the catalysts.

Among various fundamental reactions tautomeric transformation has immense implications from biological[1-2] and atmospheric[3-5] viewpoints. Tautomerization is essentially an intra-molecular hydrogen transfer reaction irrespective of the classes to which it belong e.g. keto-enol, lactum-lactim, amine-imine or amide-imidic acid. As a result, species known to catalyze tautomerization are always bifunctional in nature; acting both as proton donors and acceptors. The effects of a varied class of catalysts on tautomerization have been extensively examined[3-11] but majority of these works prioritizes one single aspect of the reaction; facilitation of tautomerization by lowering the activation barrier. On careful examination of these results, interesting observations are found that can be extrapolated and organized to provide new trends useful from practical point of view. In every report till date, without exception, the catalysts were found to stabilize two different tautomeric forms to different extents and consequently varying their relative stabilities. Moreover, in studies involving more than one species catalyzing a particular tautomeric transformation, although not highlighted there explicitly, the relative stabilities of the tautomers varied with catalysts.[6,11] An area still open for exploration is finding a technique to control the tautomeric distribution by stabilization of any one tautomeric form. Here we report a very simple approach of controlling the relative energy of two tautomeric forms as well as drastic lowering of the barrier of tautomeric interconversion by changing easily available organic acids and bases as catalysts in a selective fashion.

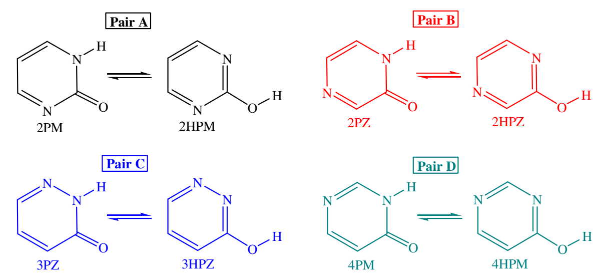

Fig. 1 Four sets of amide-imidic acid tautomeric pairs. The colour codes shown here, i.e. Black: Pair A; Red: Pair B; Blue: Pair C; Green: Pair D, has been followed throughout.

In this context, four different isomeric amide-imidic acid pairs, such as 2-pyrimidinone (2PM) ↔ 2-hydroxypyrimidine (2HPM) (Pair A), 2-pyrazinone (2PZ) ↔ 2-hydroxypyrazine (2HPZ) (Pair B), 3-pyridazinone (3PZ) ↔ 3-hydroxypyridazine (3HPZ) (Pair C) and 4-pyrimidinone (4PM) ↔ 4-hydroxypyrimidine (4HPM) (Pair D) were chosen for the investigation (Fig. 1). These four tautomeric pairs possess a number of features useful for the abovementioned nature of study. Firstly, they all exhibit amide-imidic acid [–NH–CO– ↔ –N=C(OH)–] tautomerism, which is an important process in enzyme catalyzed reactions and in storage of DNA information.[12-15] Secondly, the geometry of the tautomeric centres change very little from one pair to another as they are always part of six-member heterocyclic rings. This removes any untoward complicacies arising out of geometrical or steric factors. Finally, as shown by Galvão *et al.*,[16] these four sets of tautomeric pairs exhibit a varied tautomeric distribution. Amide form is favoured for Pair C whereas imidic acid form is preferred for Pair A and B. For Pair D, the two tautomers are separated by very small energy difference between them. Further, all these tautomeric pairs intimately mimic the hydrogen bonding sites of the pyrimidine bases present in nucleic acids. 4PM-4HPM

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† Footnotes relating to the title and/or authors should appear here.
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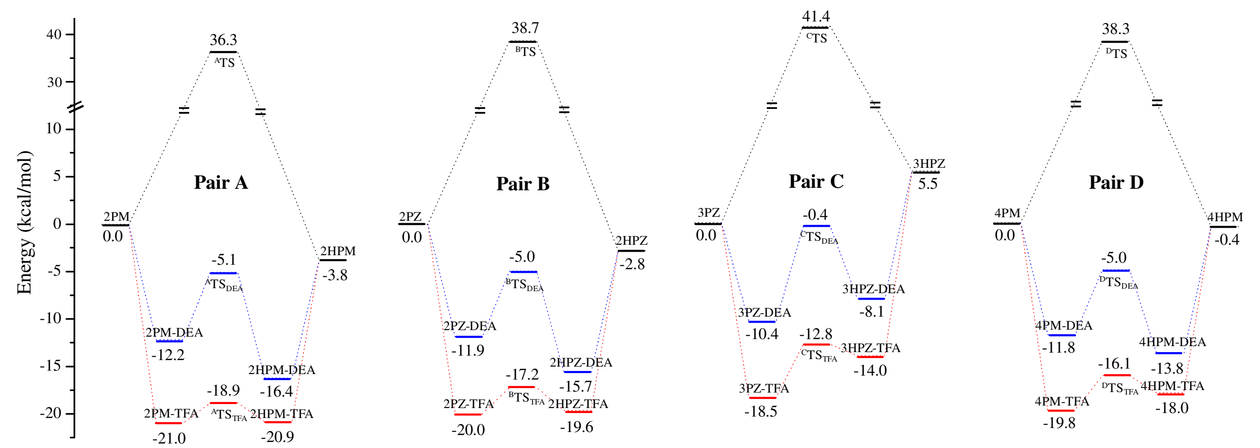

Fig. 2 Tautomeric behaviour of four sets of tautomeric pairs (Black) and effects of TFA (Red) and DEA (Blue) on them as predicted by calculations performed at M06-2X/aug-cc-pVTZ level. $^{X}TS_{Y}$ stands for transition state for 'Pair X' with catalyst 'Y'.

pair (Pair D), in particular, has been subjected to investigation through both experimental[17,18] and theoretical[16-18] methodologies with the goal of elucidating the conformational and tautomeric preferences of the pyrimidine bases.

The calculations were carried out using hybrid functional M06-2X included in density functional theory (DFT) in conjunction with aug-cc-pVTZ basis set (details given in ESI). Recent studies have shown that this level of theory is a good choice for studying hydrogen transfer reactions catalyzed by bifunctional catalysts including carboxylic acids.[4,19] For pair A, B and D, imidic acid forms were found to be more stable than amides by 3.8, 2.8 and 0.4 kcal/mol, respectively, whereas for Pair C, calculation predicted the amide form to be more stable by 5.5 kcal/mol (Figure 2 and Table S1). Thus at room temperature imidic acid forms of Pair A (2HPM) and B (2HPZ) and amide form of Pair C (3PZ) would exist almost exclusively while both tautomeric forms of Pair D would coexist in substantial amount. The activation barriers were found to be 40.1, 41.5, 41.4 and 38.7 kcal/mol when calculated from the corresponding more stable tautomers of Pair A, B, C and D, respectively (Table S2).

In their work on formamide-formamidic acid tautomerization, Constantino et al.[6] used HF and NH_3 as acidic and basic catalysts, which changed the energy difference between the two tautomeric forms from 12.5 kcal/mol to 10.9 kcal/mol and to 9.2 kcal/mol, respectively. In this work, a carboxylic acid and an amine base, namely trifluoroacetic acid (TFA) and diethyl amine (DEA), respectively, have been chosen as catalysts to investigate the catalytic effect on the potential energy profile of all four pairs (Fig. 2). The motive behind choosing these particular acid and base as catalysts is discussed later.

In absence of catalyst, the imidic acid forms of Pair A and B were exceedingly preferred over the amide forms. When TFA binds with both the tautomers, amides become marginally preferred over the other by 0.1 and 0.4 kcal/mol for Pair A and B, respectively. On the other hand, when bound to DEA, the imidic acid tautomers become even more stable and the energy difference with amide forms were found to be 4.2 and 3.7 kcal/mol, respectively. This result indicates that the intrinsic tautomeric equilibria of Pair A and B that were greatly inclined towards the imidic acid forms, could be profoundly altered by the use of TFA, which sufficiently populates both the amide and imidic acid forms at ambient condition. In contrast, DEA enhances the stabilization of the imidic acid form exclusively.

The inherent energy difference between two tautomeric forms of Pair D is very small. Here binding with TFA stabilizes the amide form and makes it stable by 1.8 kcal/mol compared to imidic acid form, whereas DEA stabilizes the imidic acid form such that it becomes 2.0 kcal/mol more stable than amide form. Thus, from a condition where both tautomers are appreciably populated, TFA and DEA separately stabilizes amide and imidic acid forms, respectively, to such extents that they would exist almost exclusively at ambient temperature. The optimized geometries of all nine species corresponding to Pair D, which have been mentioned in Fig. 2, are shown in Fig. 3.‡

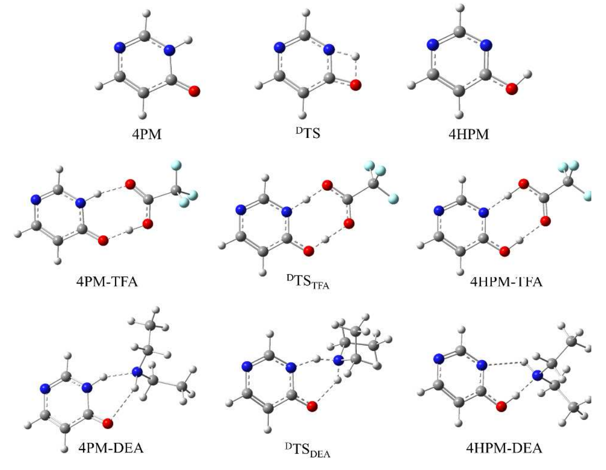

Fig. 3 Optimized geometries of monomeric, TFA- and DEA-catalyzed species for Pair D calculated at M06-2X/aug-cc-pVTZ level of theory‡

In case of Pair C, none of the two catalysts could reduce the original energy difference of 5.5 kcal/mol between two tautomers to an extent that changes their relative population by noticeable amount. Both of them, nevertheless, made the energy difference smaller: TFA reduced it to 4.5 kcal/mol and DEA to 2.3 kcal/mol.

Looking at the four cases collectively, it can be inferred that TFA and DEA turn out to be good choices as catalysts to promote population of amide and imidic acid forms, respectively. This inference is reinforced by the results obtained from calculations done at different levels and is discussed in detail later.

Having said all these, one must accept that this radical change in relative stabilities is anything but practical unless the interconversion process becomes facile under ambient condition. A simultaneous drop off in the activation barrier is the single most important factor for efficient conversion of one tautomeric form into the other. The uncatalyzed barrier heights, which were found to be in the close vicinity of 40 kcal/mol, diminish very sharply in presence of TFA and DEA. The activation energies of catalyzed reactions calculated with respect to both tautomeric forms were found to be in the range of 1-6 kcal/mol when TFA was used and 7-11 kcal/mol with DEA indicating the former being more efficient as catalyst.

It was evident from the earlier discussions that TFA and DEA as catalysts have specific roles to play in altering tautomeric distribution. TFA is useful in transforming imidic acid into amides and hence the barrier height with respect to imidic acid forms would dictate how fast that conversion could take place. Similarly DEA finds its use in transforming amides into imidic acids and hence tautomeric barrier with respect to amides would determine its efficiency as catalyst. For Pair A and B, TFA catalyzed imidic acid to amide transformation involves activation barriers of only 2.0 and 2.4 kcal/mol respectively. As DEA stabilizes the already stable imidic acid forms, it finds no use as catalyst for altering tautomeric equilibria in these two cases. Nevertheless, DEA would play a very important role in transforming amide-imidic acid mixture of Pair D into predominantly imidic acid form and the concerned activation barrier for that process is 6.8 kcal/mol when calculated from imidic acid side. Similarly TFA transform the mixture to exclusively amide form involving a barrier of only 1.9 kcal/mol from amide side. Further all the transition state energies of both TFA and DEA catalyzed reactions have lower energy than either of the free amide or imidic acid tautomers. Hence, the energy required to cross the barrier could be obtained from the energy released during binding of reactants with the catalysts. If the whole process could be carried out adiabatically, no external energy would be needed at all for this selective transfer of tautomeric population.

In addition, electronic energies of all the species shown in Fig. 2 were calculated at MP2/aug-cc-pVTZ level in order to benchmark the accuracy of the DFT energies presented above. The results (Fig. S1) reinforce the prediction made by DFT calculations. Moreover, a free energy profile using M06-2X/aug-cc-pVTZ level of theory was also constructed (Fig. S2) for comparisons between intra- and inter-molecular reactions occurring during uncatalyzed (monomeric) and catalyzed (dimeric) transformations, respectively. It is noteworthy, that incorporation of the entropic factors did not alter the trend shown by the two catalysts on both relative energies and barrier heights.

Once the opposing effects of TFA and DEA on the relative stabilities of two tautomeric forms were established, the foremost task was to ensure whether it was a one off incident or a general property of the class of compound they belong to. For this purpose three more carboxylic acids namely acetic acid (AA), formic acid (FA) and monofluoro acetic acid (MFA), and same number of amine bases i.e. ammonia (AM), methyl amine (MA) and dimethyl amine (DMA) were chosen. We confined our choice to simplest carboxylic acids and amine bases covering an appreciably wide range of pK_a values starting from 0.52 to 10.84 (Table S3). This was done purposefully for reasons that warrant a brief discussion here. Geometry of the reaction centre for the acid and base catalyzed tautomerization (Fig. S3) suffer minimum perturbation as acid and base strengths are varied by changing the side chains only. Hence the choice of catalysts minimizes geometrical influence on their efficiency. Further, catalysts containing aromatic side chains and d-group elements were excluded so that remote electronic effects could be avoided.

The effects of all the eight catalysts on relative stabilities of the tautomers and activation barriers are shown in Fig. 4 and 5, respectively. In both these figures the catalysts are arranged along X-axis in increasing order of pK_a values, i.e. acidity decreases and basicity increases as one traverse the full length of X-axis from left to right. This arrangement enables to appreciate the effects of all eight catalysts with monotonic change in acidity and basicity.

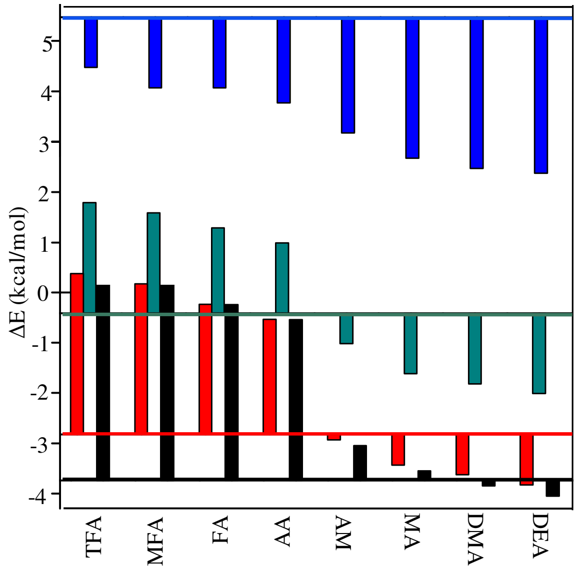

Fig. 4 Effect of catalysts on relative energies (ΔE = $E_{\text{imidic acid}}$-E_{amide}) for the four sets of tautomeric pairs calculated at M06-2X/aug-cc-pVTZ level; Black: Pair A; Red: Pair B; Blue: Pair C; Green: Pair D. Horizontal lines of corresponding colours represent the respective ΔE in absence of catalysts. For related values see Table S4

Fig. 4 reveals that for each of the four tautomeric pairs, the relative energies of imidic acids with respect to amides are highest for TFA and lowest for DEA. The values for the six other catalysts show a monotonic decrement within the limits of TFA and DEA with increasing basicity or decreasing acidity. This information is very important as it would not only allow profound alteration in the tautomeric distribution using a strong acid or base, but also variation to lesser extents by employing a suitably chosen milder acid or base. In fact, it would permit the tautomeric distribution to be tuned by regulating the strength of catalysts. Fig. 5 shows that tautomeric barriers for all four pairs decrease monotonically with increasing strength of both acid and base catalysts. The effect of acids is more prominent compared to bases, but the trends of

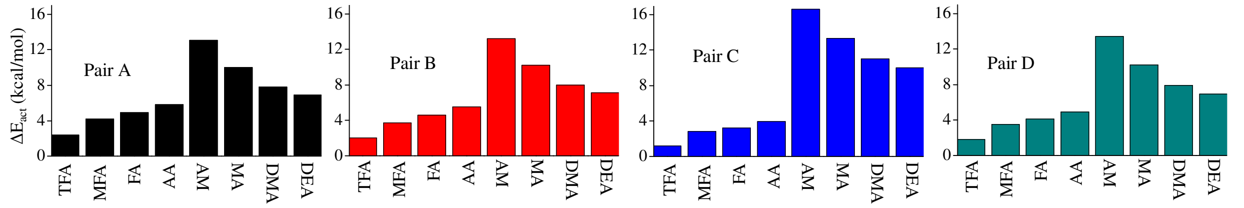

Fig. 5 Activation energies of tautomerization (ΔE_{act}) in presence of catalysts for the four sets of tautomeric pairs calculated at M06-2X/aug-cc-pVTZ level. Barrier heights for acid and base catalysts have been calculated with respect to imidic acid and amide forms, respectively. For related values see Table S5.

reduction of activation energy remain same for both the acid and base catalysts. A systematically variable transition energy by varying catalysts could prove to be a powerful tool when the goal is to adjust the reaction rate without changing any external condition like temperature. Further, relative free energies of imidic acids to amides and free energies of activation for the four pairs in presence of all eight catalysts were also calculated (Table S6 and S7) and they, too, reflect the same trends discussed above.

It is evident from the above results that there exists a systematic dependence of both relative stabilities and barrier heights on strength of the catalysts. Our final goal was to investigate the explicit form of that dependence in quantitative manner. Theoretical evaluation of deprotonation energy (DPE) and proton affinity (PA) is a well acknowledged method for determination of strength of acids and bases, respectively.[20-22] DPE is defined as the energy required to remove the acidic proton ($AH \rightarrow A^- + H^+$) and PA as the energy released during the attachment of a single proton ($B + H^+ \rightarrow BH^+$). As a proton possesses zero electronic energy, hence

$$DPE = E(A^-) - E(AH)$$

and

$$PA = E(B) - E(BH^+)$$

The above two equations indicate that acidity increases with decreasing DPE, while basicity increases with increasing PA values. The DPE values of AA, FA, MFA and TFA calculated at M06-2X/aug-cc-pVTZ level are 353.9, 350.0, 343.5 and 328.9 kcal/mol, respectively and the PA values of AM, MA, DMA and DEA are 209.5, 220.7, 228.2 and 234.4 kcal/mol, respectively.

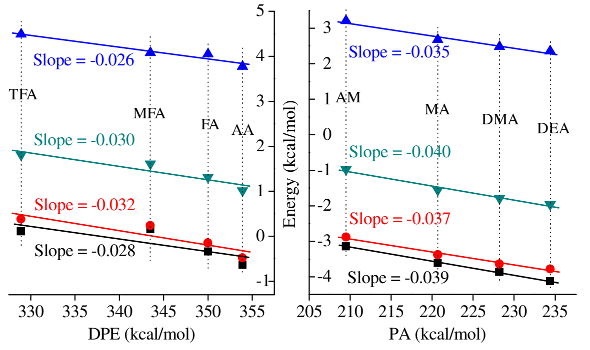

Fig. 6 Variation in relative energies against DPE and PA calculated by M06-2X/aug-cc-pVTZ level of theory; Black: Pair A; Red: Pair B; Blue: Pair C; Green: Pair D.

Relative energies of imidic acids compared to amides and tautomeric barriers for all four pairs were plotted against DPE and PA values of the eight catalysts and are shown in Fig. 6 and 7, respectively. From the two figures it is clearly evident that the relative stabilities and barrier heights exhibit a linear dependence on the acid and base strength with very little deviation of individual points from the best fitted line. Moreover, if the slopes of the individual linear fits are compared, they too, fall within a very small range. In Fig. 6, the slopes of relative stabilities vs. DPE and PA are within 0.026-0.032 and 0.035-0.040, respectively. The slopes of barrier heights vs. DPE and PA in Fig. 7 are in the range of 0.10-0.13 and 0.25-0.27, respectively. The above observation implies that this dependence is almost independent of the intrinsic tautomeric behaviours of the four pairs.

It was shown by Sanchez *et al.*[18] that the theoretically predicted relative energies of the tautomeric forms of 4PM depends heavily on the method used and two different methods can predict opposite trend altogether. This result makes it mandatory to check whether this dependence is exhibited by all four tautomeric pairs we have studied. Calculations were carried out at 6-311++G(d,p) basis set using two more DFT functional, B3LYP and B97D, beside M06-2X level of theory. The results were, as expected, different from that of M06-2X/aug-cc-pVTZ (Table S1). The amide tautomers of Pair C and D were predicted to be more stable in B3LYP/6-311++G(d,p) level while B97D/6-311++G(d,p) level predicted amide as the preferred tautomer for all four pairs. The result of M06-2X/6-311++G(d,p) level of calculation departed very little from that of M06-2X/aug-cc-pVTZ calculation.

Next, the goal was to see whether the trends shown by catalysts that have been discussed in the preceding sections also get affected by the different levels used. The outcome of this particular investigation would ensure whether the results obtained are indeed the intrinsic nature of the studied tautomeric equilibria, or merely theoretical artefact. Hence, all the calculations with the eight different catalysts for the four tautomeric pairs were carried out using the three abovementioned levels of theory. A critical analysis of this study shows that if these results are classified purely based on the relative energies of the two tautomeric forms, they barely show any dependence on either tautomeric pair or level of theory. Moreover some interesting observations were obtained as discussed hereafter.

When the calculated energy differences between the uncatalyzed tautomers belonging to any of the four pairs were found within 0.5 kcal/mol, regardless of which tautomeric form is more stable, TFA always makes amide more stable compared to imidic acid whereas DEA does exactly opposite. Within the range of 1-4 kcal/mol, two different scenarios are observed. When the amides are more stable than the imidic acids, the acid catalysts maintain the trend and TFA, the strongest one, always makes the amide forms even more stable. The basic catalysts, on the other hand, tend to minimize the

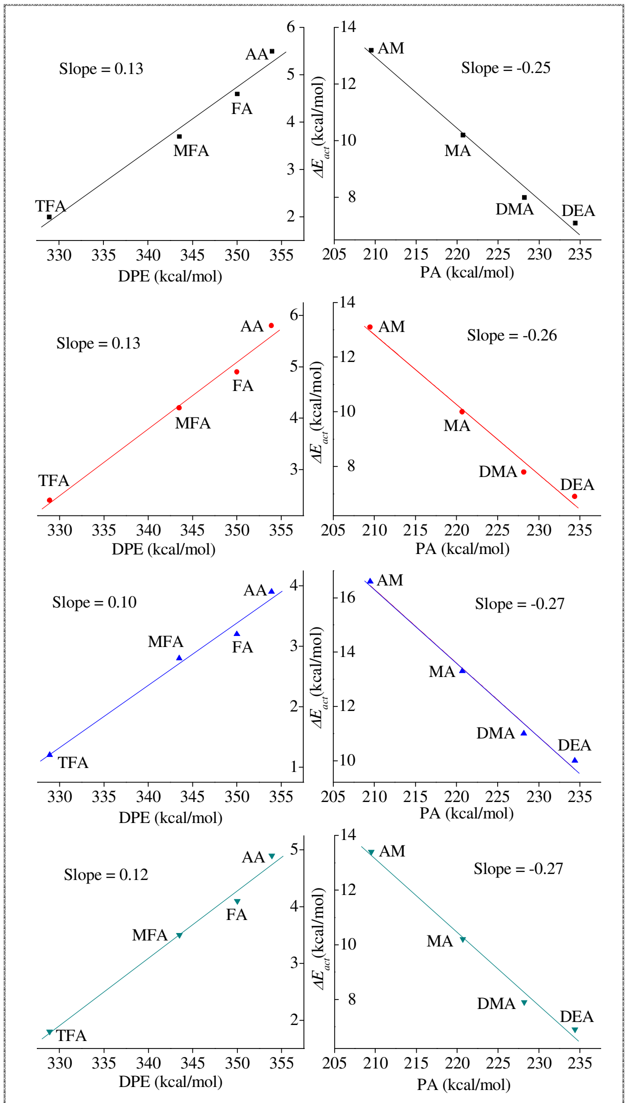

Fig. 7 Change in barrier heights against DPE and PA calculated by M06-2X/aug-cc-pVTZ level of theory. From top to bottom: Black: Pair A; Red: Pair B; Blue: Pair C; Green: Pair D.

energy difference and DEA, the strongest base, acts most effectively in doing so. When the imidic acid form is predicted to be more stable, then it is the basic catalysts that try to maintain the trend and the acid catalysts diminish or revert the trend. Once the uncatalyzed energy difference between two tautomers becomes more than 5 kcal/mol, which happens only for Pair C and is of very little practical importance, all the acidic and basic catalysts were found to reduce the energy difference. The trends discussed above are shown in Fig. S4-S6 and the related values are enlisted in Table S8-S10.

Lowering of tautomeric barrier, too, showed very similar trend for all the theoretical levels (Figure S7-S9 and Table S11-S13). The absolute values of the transition barriers for catalyzed reactions always showed monotonic decrement as the acidity and basicity of the catalysts were increased, without any exception. TFA and DEA always came out to be the best among the chosen acids and bases, respectively, in reducing the barrier. Thus, as a whole, it could be inferred that the effects shown by the acid and base catalysts on the relative energies of tautomeric forms and activation barriers for tautomeric interconversion are almost independent on the level of theory used to compute them. They solely depend on the original relative energies of the tautomers.

Conclusions

This study reveals a few hitherto unidentified trends and findings that can provide a simple yet efficient technique to alter and also tune tautomeric distribution under ambient condition by use of carboxylic acids and amine bases as catalysts. The relative energies of the two tautomeric forms showed systematic dependence on the acidity and basicity of the catalyst species. The acids were found to stabilize the amide forms more compared to the imidic acid forms whereas the bases did exactly opposite. The degree of this additional stabilization of one tautomeric form over the other showed a monotonic dependence on the acidity and basicity of catalysts. Moreover, the activation barriers were extensively reduced by the catalysts to such level that tautomeric transformation would be possible at ambient temperature. Catalyzed barriers, too, were found to depend on the acidity and basicity of the catalyst; the barrier height successively decreases with increasing acidity and basicity. Both relative stabilities and barrier heights were found to be linearly dependent on the strength of catalysts expressed as deprotonation energy (acids) and proton affinity (bases). Calculations were carried out at four different levels and they predicted different trend of relative energies between two tautomeric forms. But the effects exerted by the catalysts on the tautomeric behaviour were found to be independent of the levels of theory employed; it was only the original uncatalyzed energy difference between two tautomers that determined the effect of catalysts. As a whole, the results reported here could open up ways to control and manoeuvre the tautomeric distribution in ways that fit our need.

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Notes and references

‡Geometries of all the species optimized at M06-2X/aug-cc-pVTZ level are shown in Fig S10

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